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THE ETIOLOGY OF AMYLOID DISEASE

WITH A NOTE ON EXPERIMENTAL RENAL AMYLOIDOSIS

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MINNEAPOLIS

Many theories have been proposed to account for the etiology of the type of amyloidosis which is regarded as secondary to chronic diseases and is characterized by large deposits of typical amyloid substance in the spleen, kidneys and liver. Less is known about the type of amyloidosis which is apparently primary and is characterized by the presence of atypical amyloid substance in smooth and striated muscle. For the most part hypotheses have been based on speculation by numerous authors who have reported cases, but a number of experimental studies have been made. Among the more important theories are those which claim that amyloidosis is due to: (*a*) a general disturbance of protein metabolism (Beneke,[1] Kuczynski[2] and Grayzel[3]), (*b*) an antigen-antibody union and precipitation (Letterer,[4] Loeschcke,[5] Primgaard[6] and Lucke and Markley[7]), (*c*) an absorption of protein (Cruz[8]), (*d*) a disturbance or abnormality of the reticulo-endothelial system (Smetana,[9] Arndt,[10] Doerken[11] and Stoeber[12]), (*e*) hyper-

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1. Beneke, R.: Centralbl. f. allg. Path. u. path. Anat. **33**:240, 1922.
2. Kuczynski, M. H.: Virchows Arch. f. path. Anat. **239**:184, 1922.
3. Grayzel, H. G.; Jacobi, M.; Warshall, H. B.; Bogin, M., and Bolker, H.: Arch. Path. **17**:50, 1934.
4. Letterer, E.: Zentralbl. f. inn. Med. **47**:417, 1926; Virchows Arch. f. path. Anat. **293**:34, 1934.
5. Loeschcke, H.: Beitr. z. path. Anat. u. z. allg. Path. **77**:231, 1927.
6. Primgaard, T.: Virchows Arch. f. path. Anat. **274**:111, 1929.
7. Lucke, B., and Markley, L. A.: Proc. Soc. Exper. Biol. & Med. **25**:642, 1928.
8. Cruz, D. J.: Frankfurt. Ztschr. f. Path. **41**:250, 1931.
9. Smetana, H.: J. Exper. Med. **45**:619, 1927.
10. Arndt, H. J.: Verhandl. d. deutsch. path. Gesellsch. **26**:243, 1931.
11. Doerken, E.: Virchows Arch. f. path. Anat. **286**:487, 1932.
12. Stoeber, E.: Deutsches Arch. f. klin. Med. **176**:643, 1934.

globulinemia (Koref,[13] Magnus-Levy [14] and ourselves [15]) and (*f*) hyperproteinemia and disturbance of the reticulo-endothelial system (Steinhaus [16] and Morgenstern [17]). The subject has been thoroughly discussed by Letterer.[4]

The first hypothesis mentioned, namely, that amyloidosis is due to a general disturbance of protein metabolism, is vague and was suggested principally by the protein nature of the deposited substance. There is little to support the concept of antigen-antibody union and precipitation, since amyloid disease may follow diseases or experimental procedures which do not involve immunization processes; for example, it may occur after feeding large amounts of casein to mice or after the injection of inorganic substances. The theory of disturbance of the reticulo-endothelial system is plausible, since in secondary amyloidosis the substance is deposited chiefly in organs richest in reticulo-endothelial tissue.

The theory of an increased protein content in the blood stream together with reticulo-endothelial abnormality was proposed in substance in 1855 by Rokitansky,[18] who expressed the belief that the presence of amyloid substance is due to infiltration or deposit of an abnormal material from the blood. The following observations support this view: Amyloid disease often follows chronic diseases, such as malignant conditions and multiple myeloma, and chronic infections, such as tuberculosis and syphilis, which are known to be associated with long periods of hyperglobulinemia. Amyloid disease was noted in a high percentage of horses repeatedly inoculated with antigen for the production of immune serums.[19] Injection of vaccine has long been known to provoke an increase in the globulin content of the blood,[20] and in consequence thereof we [15b] recently reported observations on a patient who received forty-four injections of vaccine for the treatment of arthritis and subsequently died of amyloid disease, which we believed to be the result of the prolonged hyperglobulinemia induced by the injections. Many other substances when repeatedly injected into animals over long periods induce the deposition of amyloid substance.[21] Amyloid disease has been induced also by the feeding of excessive amounts of protein,

13. Koref, O.: Med. Klin. **20**:1243, 1924.

14. Magnus-Levy, A.: Ztschr. f. klin. Med. **126**:62, 1933.

15. Reimann, H. A., and Eklund, C. M.: (*a*) Arch. Path. **20**:163, 1935; (*b*) Am. J. M. Sc. **190**:88, 1935.

16. Steinhaus, F.: Ztschr. f. klin. Med. **45**:375, 1902.

17. Morgenstern, Z.: Virchows Arch. f. path. Anat. **259**:698, 1926.

18. Rokitansky, C., cited by Kretzschmar, P. H., and Westbrook, B. F.: Proc. M. Soc. County Kings **5**:343, 1880.

19. Arndt.[10] Doerken.[11]

20. Langstein, L., and Mayer, M.: Beitr. z. chem. Physiol. u. Path. **5**:69, 1904.

21. References to papers describing these experiments have been given previously.[15b]

which is known to result in an increase in the amount of globulin. The participation of impairment of the reticulo-endothelial system, although highly probable, has been difficult to demonstrate. When histologic evidence of cellular changes is observed it is not possible to state whether the changes were the cause of or were caused by the amyloid deposits.

Letterer [4] has also noted a close relationship between changes in the globulin content of the blood and amyloid disease and has cited the work of Schmiedeberg, who found the hydrogen, carbon and nitrogen contents of serum globulin and of amyloid substance to be similar. This observation, however, does not appear to us to be of great significance. Letterer studied the blood globulin in mice with amyloid disease. Under the disadvantage of working with minute amounts of blood, he found that the globulin content was increased in animals which had been given injections, in which amyloid was not observed, and that it was decreased in animals with amyloidosis. He concluded therefrom that the globulin was precipitated as amyloid during the process of its formation. Other observers have reported a failure to obtain significant results. Jaffé [22] observed no change in the protein content of the blood in mice with amyloid disease. Achard and his associates,[23] working with dogs, noted no change in the total protein content during amyloidosis but observed that the albumin content dropped and that the globulin content usually increased. Because of the inconclusiveness of these experiments, the problem was reinvestigated with rabbits, which were observed for many months, until death occurred.

METHODS

After the blood protein level had been determined in five rabbits, each animal was given an intramuscular injection of 5 cc. of a 10 per cent solution of sodium caseinate three times a week. Injections were continued until shortly before death. Two rabbits were kept as controls in similar cages and were given similar food. The protein content of the plasma was measured weekly during the first four months and then twice a month. The albumin and globulin values of the blood were determined by the method of Medes [24] and the fibrinogen value by the method of Cullen and Van Slyke.[25]

Immediately after death necropsy was performed in each case, and sections of tissue were tested with aqueous solution of iodine U.S.P. and double-normal sulfuric acid, congo red and methyl violet.

RESULTS

The rabbits were observed until death from amyloidosis occurred. It was not possible, therefore, to determine when amyloid was first deposited. The first

22. Jaffé, R. H.: Arch. Path. **1**:25, 1926.

23. Achard, C.; Bariéty, M.; Codounis, A., and Hadjigeorges, E.: Compt. rend. Soc. de biol. **108**:702, 1931.

24. Medes, G.: Am J. Clin. Path. **3**:439, 1933.

25. Cullen, G. E., and Van Slyke, D. D.: J. Biol. Chem. **41**:587, 1920.

evidence of illness was loss of weight and ruffled fur, which were usually noted two or three months preceding death. The weight of each animal diminished to two thirds or one half of the original weight. Two animals died in eight months, one in eleven months and one in thirteen months, and the fifth, which behaved somewhat differently, lived for seventeen months after the first injection.

The changes in the blood protein content observed in each of the rabbits were remarkably uniform. The globulin content in each case rose above the normal level approximately two weeks after the first injection and continued to rise until the peak was reached, from four to six weeks later, at which time the amount was from two to four times the normal value. The amount present remained at those high levels during the first four or five months and then declined but remained well over normal until death occurred. No significant changes were noted in the fibrinogen levels, which were in each case plotted with the globulin values.

The albumin content remained constant, though slightly below normal, for four or five months and then dropped rather rapidly until one half or less of the initial amount remained. The total protein content rose during the second week, reached the highest level in from four to six weeks and remained high fairly constantly for four or more months, when the decrease of the globulin content and especially of the albumin content brought the level somewhat below normal. During the last few months of life the relationship of the plasma protein levels resembled that found in nephrosis in man. The results are graphically illustrated in figures 1 and 2.

Rabbit 5 lived for seventeen months after the first injection. In this animal the globulin value increased, as usual, to 4.2 Gm. per hundred cubic centimeters in six weeks and then gradually diminished to twice the normal amount until the twelfth month. The amount then increased again until shortly before death, when 5 Gm., or four times the normal amount, was present. The albumin content diminished by about one half in five months and remained at that level. The fibrinogen value rose slightly. One week before death congo red in a dose of 1.75 mg. per kilogram of body weight was injected intravenously. All the dye was removed from the blood stream after four minutes. The test was repeated in twenty-four hours, and after four minutes the serum was only faintly pink.

One control animal was still healthy at the end of twenty months; the other died at the end of twelve months, with no evidence of amyloidosis.

In four rabbits the bulk of amyloid substance was deposited in the kidneys; only traces were observed, microscopically in the spleen and liver. In rabbit 5, which outlived the others by several months, the spleen and liver were extensively involved. Histologically in each case the deposited amyloid substance was deeply stained with congo red; it stained in the characteristic manner with methyl violet and became bluish black when the iodine-sulfuric acid test was applied.

Observations on Renal Amyloidosis.—Each of the five rabbits studied died with signs and symptoms of uremia caused by extensive amyloidosis of the kidneys. Previous observers also have reported the experimental production of renal amyloid disease, Bailey [26] by the repeated injection of colon bacilli and Butt [27] by injections of manganese chloride. Recently Dick and Leiter [28] obtained similar results by using hemolytic streptococci.

In each of the rabbits that we studied the total blood protein level rose above normal after the first few injections of sodium caseinate and reached

26. Bailey, C. H.: J. Exper. Med. **23**:773, 1916.

27. Butt, E. M.: Arch. Path. **10**:859, 1930.

28. Dick, G. F., and Leiter, L.: Tr. A. Am. Physicians **50**:135, 1935.

its maximum in about six weeks, as previously described. After four or five months the level began to diminish, owing chiefly to a diminution in the albumin content and to a lesser extent to the lowering of the globulin content, although

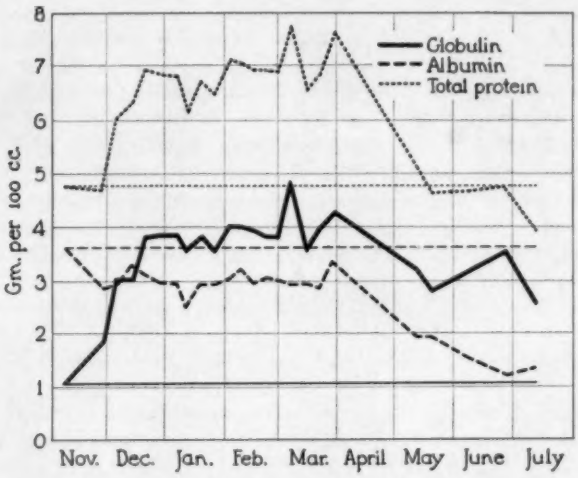

Fig. 1.—Blood protein levels of rabbit 3 observed until death occurred from renal amyloidosis and uremia, eight months after the first injection of sodium caseinate. The globulin content increased rapidly to more than two or three times the normal amount and remained high throughout the experiment. The albumin content diminished after the fifth month, causing a lowering of the total protein level.

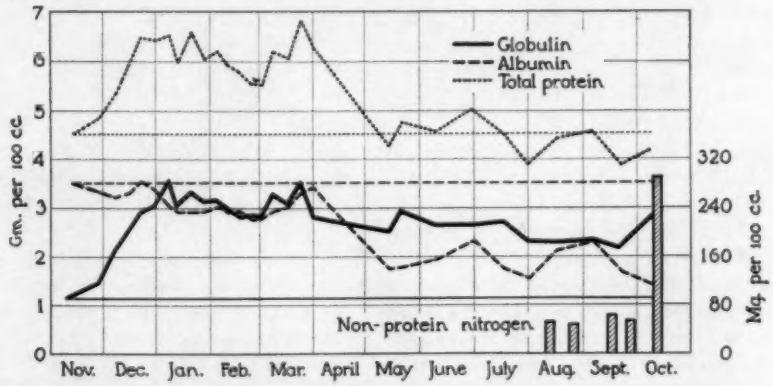

Fig. 2.—Blood protein and nonprotein nitrogen levels of rabbit 2, observed for eleven months. The globulin content increased rapidly during the first few weeks and remained above the initial level throughout the experiment. The albumin content diminished, especially after the fifth month, thereby lowering the total protein level. Albuminuria was first noted in April. The terminal stage was marked by a great increase in the nonprotein nitrogen content.

the latter still remained well above the normal level. The appearance of marked albuminuria in four of the rabbits at about that time suggested a causal relationship. The depletion of the blood protein content was believed to be due to the development of amyloid deposits in the kidney to such an extent as to interfere with renal function and permit an escape of albumin from the blood through the injured glomeruli. It was not possible to determine precisely when amyloid substance was first deposited in the kidney, since the organs were examined only after the rabbits died, from three to thirteen months after the first evidence of renal impairment was noted.

In rabbit 1 albuminuria was first noted about seven months after the first injection of sodium caseinate, when the blood protein had declined considerably in amount. Four months later, or six weeks before death, the degree of albuminuria was recorded as 3+. On one occasion 0.66 Gm. of protein was excreted in twenty-four hours, 74 per cent of which was albumin and 26 per cent globulin. Granular, hyaline and waxy casts were present in the urine. The nonprotein nitrogen content of the blood was at a normal level (40 mg. per hundred cubic centimeters) until two months before death, when it increased rapidly from 56 to 193 mg. and reached 398 mg. during the terminal stage. The blood pressure at that time was 120 systolic and 74 diastolic. The eyegrounds appeared to be normal. At necropsy the kidneys were pale and enlarged, weighing 16 Gm. and 15 Gm., respectively, which was twice the weight of kidneys of a normal rabbit of similar weight.

Essentially similar observations were made on rabbits 2, 3 and 4. In rabbit 2 the nonprotein nitrogen measured 292 mg. per hundred cubic centimeters a few days before death. The curves of the protein and the nonprotein nitrogen levels of the blood of this animal are illustrated graphically in figure 2.

In rabbit 5 the total protein content of the blood was reduced to a level below normal five months after the first injection of sodium caseinate. The nonprotein nitrogen level at that time was normal (40 mg.). Albuminuria, recorded as of 2+ and 3+ degree, appeared in the fourteenth month; the nonprotein nitrogen content had increased to 70 mg. That was nine months after the decrease in the total protein content of the blood had occurred. In this animal, therefore, the diminution of the albumin content of the blood could not be ascribed to a loss of albumin by way of the urine. In the sixteenth month 0.9 Gm. of protein, consisting of 70 per cent albumin and 30 per cent globulin, was excreted in the urine in one twenty-four-hour period. The specific gravity of the urine was fixed at 1.013, whether a full diet was given or whether fluid and food were withheld for twenty-four hours. The nonprotein nitrogen content of the blood was 116 mg. per hundred cubic centimeters. During the last months of the animal's life the left kidney was enlarged and palpable. Congo red injected intravenously was completely removed from the blood within four minutes. During the seventeen-month period of observation the rabbit's weight dropped from 2,600 to 1,300 Gm. At necropsy extensive amyloidosis of the kidneys was evident; one kidney weighed 14.7 Gm. and the other 9 Gm. They were stained bright pink by the congo red injected a week before death. On the cut surfaces numerous dots about 0.25 mm. in diameter, which probably represented the amyloid substance packed in the glomeruli, were scattered in the cortex. In the medulla numerous pink striae radiated from the papillae to the cortex. The liver and spleen were not enlarged but were extensively infiltrated with amyloid substance.

Histologically (as reported by Dr. R. W. Koucky) there was almost complete replacement of the glomerular tufts by a homogeneous substance which gave

the characteristic amyloid reaction with methyl violet and Mallory's trichrome stain. Within this substance were a few spindle-shaped, compressed remnants of nuclei. The capillary spaces were usually obliterated. In other glomeruli, the nuclei and capillaries were less distorted. The glomeruli appeared to be large and crowded together, probably as the result of atrophy of the intervening tubules. There were widespread atrophy and dilatation of the tubules; several consecutive fields of the medulla were examined under the low power lens, and no tubule was observed. Where tubules were noted they showed no infiltration of amyloid substance. Casts which stained like amyloid substance were numerous in the lumens, as shown in figure 3. The stroma between the glomeruli and the persisting tubules failed to react like amyloid substance. Similar observations were noted for each rabbit.

Fig. 3.—Glomeruli infiltrated with amyloid substance. The tubules are dilated and obstructed with amyloid casts.

COMMENT

The experiments reported show that constant hyperglobulinemia induced by repeated injections of sodium caseinate preceded the development of amyloid disease in each of five rabbits. The observations lend support to the theory that chronic hyperglobulinemia is an important factor in the development of amyloidosis of the secondary type. It is assumed that during long periods of hyperglobulinemia an attempt is made by the cells, apparently designed for the purpose, to remove the excess of normal or abnormal globulin from the blood. If the amount is not too great or too persistent the excess can be disposed of successfully. However, if the cells become overwhelmed or exhausted or if

they cease to function, owing to injury or disease, excess amounts are deposited in increasing quantities until the condition is incompatible with life. Furthermore, experimental studies and clinical observations show beyond doubt that the process is reversible.[29] If the conditions responsible for the hyperglobulinemia are removed, provided the amyloidosis is not too extensive, resorption of the substance and recovery may occur.

When deposition of amyloid takes place in the kidneys to such an extent as to interfere with the normal excretion of metabolites, signs and symptoms of nephrosis or nephritis and uremia follow.[30] Albuminuria and edema appear. In the rabbits studied, histologic sections of the kidneys showed extensive destruction of glomeruli caused by the deposit of amyloid substance in the tufts. The tubules were generally dilated and plugged with amyloid. In rabbits 1 to 4 it appeared that the depletion of the albumin content of the blood was caused by the escape of abnormally large amounts of albumin through the damaged kidneys. In rabbit 5 a diminution in the amount of blood albumin was noted nine months before marked albuminuria occurred. In this rabbit, however, in contrast with the others, there was extensive infiltration of the liver and spleen, which may have been partly responsible for the diminution in the amount of blood protein. The rôle played by the liver and other cells in the formation of blood protein has been discussed elsewhere.[31] The symptoms, signs, laboratory data and pathologic changes in the kidneys of rabbits in the terminal stage of amyloidosis were typical of those noted during renal amyloidosis and uremia in man in regard to albuminuria, normal blood pressure, normal eyegrounds, retention of nonprotein nitrogen, a positive congo red test and a lowering of the blood protein level, with a marked loss of albumin and a relative or absolute increase in the amount of globulin. Edema was not noted.

The inability of several investigators [32] to associate definite or constant changes in the blood protein with amyloid disease may have been due to the development of renal amyloidosis. As shown in these experiments, in each animal extensive renal amyloidosis developed, which appeared to be heralded clinically by albuminuria and a drop in the blood protein level. It is well known that the level of blood protein, especially that of the albumin, is disturbed by certain renal lesions, and attempts to correlate changes in the blood protein with the etiology

29. Reimann, H. A.: J. A. M. A. **104**:1070, 1935. References to other papers are given in this article.

30. Bell, E. T.: Am. J. Path. **9**:185, 1933.

31. Reimann, H. A.; Medes, G., and Fisher, L.: Folia haemat. **52**:187, 1934.

32. Letterer.[4] Jaffé.[22] Achard and others.[23]

of amyloidosis late in the disease are apt to be confused. We have found reports of twenty-six cases of amyloidosis in which the blood protein level was recorded as normal or subnormal. Each of these cases was studied in the advanced stage, after renal impairment had developed and in many instances after hepatomegaly was present. Both renal and hepatic lesions are known to modify the blood protein level. It is misleading, therefore, to attempt to correlate the changes in the blood protein level found in advanced amyloidosis with the etiology of the disease. The conditions are different during the incipient period of the disease, as shown in the early period of the experiments reported here; the amount of blood protein, especially that of the globulin, was markedly above normal. In the late stage, when evidence of renal disease appeared, the total protein content was reduced below the normal level, because of the marked diminution of the albumin content.

SUMMARY

Fatal amyloidosis was induced in five rabbits by repeated injections of sodium caseinate over long periods. Soon after the beginning of the experiment hyperglobulinemia developed in each rabbit, and it persisted until death. The total protein content of blood was increased in the early period of the experiment but diminished below normal late in the course when evidence of renal amyloidosis and uremia appeared. These experiments support the view that chronic hyperglobulinemia is an important factor in the etiology of amyloidosis of the secondary type.

THE PARATHYROID GLANDS

I. A STUDY OF THE NORMAL GLAND

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While certain tumors of the parathyroid gland were being investigated it was found necessary to refer to the literature for details of normal structure. It was found that most textbooks treated the subject in a cursory fashion and that the literature as a whole presented a considerable variation as to the gross and microscopic anatomy of the normal gland. Furthermore, while many phases of the subject were excellently presented by various authors, there were many discrepancies and omissions. In an attempt to clarify the situation and to present some of the more important and interesting data this paper has been prepared.

HISTORICAL REFERENCES

Though Owen [1] (1862) described a parathyroid gland seen in the rhinoceros, Virchow [2] (1863) first noted the structure in man. Many other workers must undoubtedly have noted these bodies but apparently considered them to be lymph nodes or small masses of accessory thyroid tissue. It remained for Sandström [1] (1880) to establish definitely the parathyroid glands as structures entirely independent of the thyroid gland. He named them glandulae parathyroideae with the implication that they were thyroid cell rests. Kohn,[3] after much careful study, favored the term *Epithelkörperchen* and supported the claim that they were of independent origin.

EMBRYOLOGY

The parathyroid glands, usually four in number, grow as paired structures from thickenings of the endotherm of the third and fourth branchial pouches. Their development is entirely independent of the thyroid gland. The superior pair of glandules arise from the fourth pouches and are hence sometimes termed the "glandulae parathyroideae IV." Similarly the inferior pair are derived from the third pouches and are called "glandulae parathyroideae III." This transposition has been brought about by the fact that in the general caudal migration of

1. Quoted by Marine, D.: The Parathyroid Glands, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, vol. 1, chap. 17, p. 575.
2. Virchow, R.: Die krankhaften Geschwülste, Berlin, A. Hirschwald, 1863, vol. 3, p. 13.
3. Kohn, A.: Arch. f. mikr. Anat. **44**:366, 1895.

the branchial derivatives the thyroid and the glandulae parathyroideae IV have been outdistanced. Piersol [4] gives the following information: "It should be noted that the close association of the thymus and upper parathyroids results in a downward displacement and transposition, so that, eventually, the upper parathyroids come to lie below the original outgrowths."

The extremely close relationship of the parathyroid glands to both the thyroid and thymus (fig. 1) is accountable for the not infrequent presence of one or more of the former bodies within the tissues of the latter structures. Though thymic tissue has been found within the para-

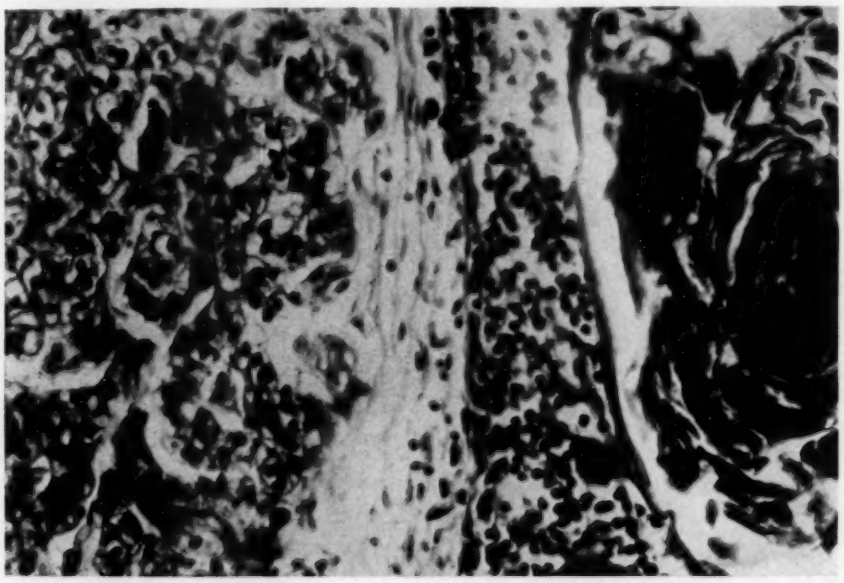

Fig. 1.—Infant parathyroid and thymus; × 800.

thyroid glands, no thyroid tissue has been observed in this location. It will be noted that the not infrequent difficulty encountered in the identification and surgical removal of these structures may often be explained on the basis of their embryologic development.

ANATOMY

Number.—The human being usually possesses four parathyroid glands, but many variations have been reported. In some cases competent workers have been unable to find more than one or two glands.

4. Piersol, G. A.: Human Anatomy, ed. 5, Philadelphia, J. B. Lippincott Company, 1916, p. 1801.

On the other hand, Erdheim [5] reported the finding of eight glandules, and in a case of gastric tetany MacCallum [5] found five pairs of these structures. Because these bodies are small, rather difficult to find and, till of late, considered to be of relative unimportance, they are seldom removed at the routine autopsy. As stated by Welti,[6] Millzner [7] and MacCallum [8] and proved by me, the number of parathyroids found at postmortem examination increases with the skill and experience of the dissector. To illustrate the variation in number of glands found by various observers the figures in table 1 are of interest.

A more detailed record of Valkanyi's [9] studies is as follows: In 70 per cent of cases four glandules were found, and in 24 per cent three glandules were present. Only two parathyroids were found in 5 per cent of cases while in 2 per cent only one gland could be discovered.

Klotz [10] and others have stressed the fact that the symmetry of distribution is far from constant and that only one glandule may be present on one side with one, two or three on the other. Failure to find

TABLE 1.—*Variation in Number of Parathyroid Glands Found by Various Observers*

Author	Number of Cases	Average Number of Glands
MacCallum [8]	64	3.2
Valkanyi [9]	225	3.6
Welti [6]	50	2.8
Pool, E. H.: Ann. Surg. **46**: 507, 1907	16	2.9
Lorin, quoted by Welti [6]	15	2.8

at least four parathyroid glands may be attributed to one or more of the following causes: (1) congenital absence of one or more of the glands; (2) inclusion of a gland within the thyroid (Lahey [11]); (3) inclusion of a gland in thymic tissue; (4) abnormal position, as between the trachea and esophagus, posterior to the esophagus (Walton [12]), in the carotid region or in the anterior mediastinum; (5) asymmetrical distribution of glands (Klotz [10] and Welti [6]); (6) lack of experience and patience of dissector.

5. Quoted by Klotz, O.: Pathological Anatomy and Histology, Endocrinology and Metabolism, New York, D. Appleton & Company, 1922, vol. 1, p. 557.

6. Welti, H.: Chirurgie des Parathyroides, in Rapport, Association français de Chirurgie, Compt. rend. XLII Congrès français de Chirurgie, Paris, Brodard & Taupin, 1933.

7. Millzner, R. J.: Anat. Rec. **48**:399, 1931.

8. MacCallum, W. G.: Brit. M. J. **2**:1282, 1906.

9. Valkanyi, R.: Presse méd. **33**:452, 1925.

10. Klotz, O.: Pathological Anatomy and Histology, Endocrinology and Metabolism, New York, D. Appleton & Company, 1922, vol. 1, p. 557.

11. Lahey, F. H.: Surg., Gynec. & Obst. **42**:508, 1926.

12. Walton, J.: Brit. J. Surg. **19**:285, 1931.

Size, Shape, Weight and Color.—In my series, which included over three hundred parathyroids from both surgical and postmortem sources, no great variation in size was noted. The length varied from 4 to 9 mm. and the diameter from 2 to 4 mm., the lower pair of glands being usually larger than the upper. Even a cursory review of the literature reveals a marked variation in the size of the glands reported as normal structures.

The size of any one gland is, to some extent at least, dependent on the total number present, the relationship being an inverse one. I believe that the congenital absence of one or more parathyroid glands may be reflected in a hyperplasia of one or more of those glandules present and hence may explain the presence of some abnormally large glands of normal histologic structure.

TABLE 2.—*Variation in the Size of Parathyroid Glands Reported in the Literature*

Author	Gland Measurements
Castleman and Mallory [21]	3 to 6 mm. by 2 to 4 mm. by 0.5 to 2 mm.
Collip, J. B.: Medicine **5**:1, 1926	3 to 15 mm. by 2 to 3 mm.
Marine [14]	6 to 7 mm. by 3 to 4 mm. by 1.5 to 2 mm.
Cowdry [18]	6 by 3 by 2 mm.

TABLE 3.—*Normal Weight of the Parathyroid Glands*

Author	Year	Average Weight, Gm.		Total Weight., Gm
		Upper	Lower	
Welsh [23]	1898	0.035		
Maranon, S., quoted by Pappenheimer and Wilens [13]	1911	0.020-0.050		0.080-0.120
Danisch, F.: Frankfurt. Ztschr. f. Path. **30**:443, 1924	1924	0.026-0.030	0.037-0.041	
Marine [14]	1928	0.020	0.035	
Aibara, S.: Tr. Jap. Path. Soc. **21**: 188, 1931	1931	.....	.....	0.067
Pappenheimer and Wilens [13]	1935	0.027	0.031-0.032	0.118

The glands are soft, flabby and inelastic, and their shape is easily altered by pressure of contiguous structures. This fact explains the frequent finding of longer flattened glandules in relation to adenomatous goiters.

Pappenheimer and Wilens [13] have recently stressed the paucity of knowledge concerning the normal weights of the parathyroid glands. A review of the literature to date is summarized in table 3.

Pappenheimer and Wilens [13] also present the following facts: The glands from females are approximately 20 per cent heavier than those from males, the difference being most apparent during the active sexual life of the female but not related to pregnancies. After the menopause there is a gradual loss of gland weight to approach that of the male. The male gland shows no change related to age. The lower pair are approxi-

13. Pappenheimer, A. M., and Wilens, S. L.: Am. J. Path. **11**:73, 1935.

mately one-fifth heavier than the upper. In my study no specific record of weights was made. If, however, there is a definite relationship between the size of the gland and its weight, and I believe this to be the case, the measurements of the glands which I studied indicate a weight variation similar to that of the glands recorded by these authors. It was also noted that the size and weight of the gland were affected by the degree of vascularity of the structure. The amount of vascular congestion was noted on gross examination and confirmed by microscopic studies. Further, because of the marked variation in fat content of the glands, the weight cannot be directly interpreted as indicative of the amount of parenchymal tissue present. The weight and size of any one gland is influenced by the following factors: (*a*) the age and sex of the patient, (*b*) in females, the stage of sexual activity, (*c*) the number of parathyroids present, (*d*) the blood content of the organ.

The fresh gland has a smooth glistening capsule which separates it from contiguous tissue. The capsular surface is of a homogeneous color, and section reveals the cut surfaces to be of a similar even color throughout. The degree of pigmentation of the glands is also variable, and in this regard my observations agree with those of Marine,[14] who stated, "The writer has gained the impression that the color tends to darken somewhat with age and that there is a vague parallelism in the degree of pigmentation of the thyroid and parathyroid." The color in infancy and childhood is a light yellowish red with a gradual darkening to a yellowish brown or reddish brown with increasing age. The change coincides with the increase in the interlobular and intracellular fat content of the gland. The degree of vascularity of the structure and the presence of blood pigment following hemorrhage also influence the color of the glandule.

Positions and Relations.—The superior pair of glands are more constant in position and are usually found on the posteromedial surface of the thyroid lobe, about the level of the junction of the upper and middle thirds. The inferior pair are most commonly found on the posterior surface of the inferior pole of the thyroid. Welti [6] has prepared an excellent account of the normal and abnormal positions in which these structures may be found, and further repetition is unnecessary.

Walton,[12] in his review of the surgical anatomy of the gland, stresses the importance of the relationship of the parathyroids to the thyroid fascia. A thorough knowledge of this relationship is essential to the understanding of the various positions assumed by the enlarged gland. This publication is readily accessible and should be of value to all interested in removal of the parathyroid glands.

14. Marine, D.: The Parathyroid Glands, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, vol. 1, chap. 17, p. 575.

Blood Supply.—My studies of the vascular supply of the parathyroid glands agree with those of Halsted and Evans [15] and are summarized as follows: The parathyroid glands have a relatively large blood supply, and both pairs usually derive nutriment from branches of the inferior thyroid artery. These vessels are easily picked up, and the identification and tracing of them often prove an excellent means of finding the glands. The superior pair of bodies may occasionally be supplied by a branch

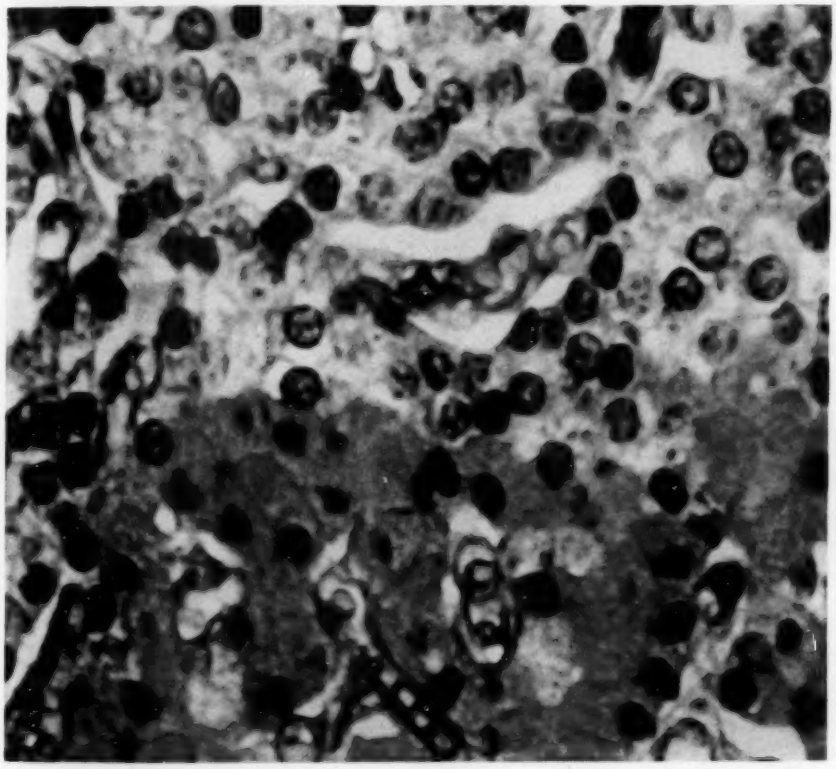

Fig. 2.—Light and dark principal cells and oxyphil cells and their intimate relation to capillaries; × 400.

from an anastomosing trunk of the superior and inferior thyroid arteries. The arteries to the glands are of the terminal or "end" type, and there is no anastomotic circulation. This type of vascular supply is in marked contrast to that found in the thyroid gland, where the anastomosis is so efficient that even ligation of a main branch fails to produce infarction. Since ligation of a parathyroid artery always causes infarction, and incautious manipulation of this structure is often followed by a similar

15. Halsted, W. S., and Evans, H. M.: Ann. Surg. **46**:498, 1907.

result, great care must be exercised in operative procedures in the region of these structures. The blood vessels enter and leave at the hilus. Almost immediately after entering the gland the artery divides into large arterioles. The latter vessels then break up into large sinusoidal capillaries which run along the trabeculae. The parenchymal cells lie immediately adjacent to the endothelium of the capillaries which ramify throughout the gland (fig. 2) and are especially prominent beneath the capsule. The main vein leaves at the hilus, has an irregular course and may enter into the thyroid, esophageal or tracheal veins.

Nerve Supply.—Rhinehart [16] states that the nerves supplying the gland are few, nonmedullated and vasomotor in type. After entering at the hilus the nerves are distributed as a delicate perivascular plexus which extends along all the fine ramifications of the vascular system. The nerves of the parathyroid are derived from the middle and inferior ganglions of the cervical sympathetic trunk.

Lymphatics.—Little is known concerning the distribution of the lymphatics in the parathyroid gland. A few small endothelial-lined spaces were seen which were apparently lymph channels, but no detailed study of these structures was undertaken.

CYTOLOGY

A study of the cytology of the parathyroid gland leaves, I believe, much to be desired with regard to fine detail of cellular structure, completeness of information and uniformity of nomenclature. Among the more constructive works available are those of Cooper,[17] Marine,[14] Cowdry [18] and Turnbull [19] and his associates.

MATERIALS AND METHODS

Through the kind cooperation of Dr. Sidney Farber of the Children's Hospital, Boston, I was able to make a detailed study of the parathyroid glands of children up to the age of 14 years. From the same source paraffin blocks of tissue fixed in Zenker's solution were secured from fourteen children ranging in age from an 8 month fetus to a child of 8 years. From the surgical files of the pathologic department of the New England Deaconess and New England Baptist Hospitals a large number of slides of parathyroid tissue were available. The autopsy service of these institutions, under the direction of Dr. Shields Warren, provided fresh normal parathyroids from over one hundred persons ranging from a 6 week embryo to adults of 70 years. The material therefore consisted of over three

16. Rhinehart, D. A.: Am. J. Anat. **13**:91, 1912.

17. Cooper, E. R. A.: The Histology of the More Important Human Endocrine Organs at Various Ages, New York, Oxford University Press, 1925, chap. 6, p. 77.

18. Cowdry, E. V.: Endocrinology and Metabolism, New York, D. Appleton & Company, 1921, vol. 1, p. 501.

19. Turnbull, H. M.: Brit. J. Surg. **19**:203, 1931.

hundred parathyroids, all of which were studied in detail and many subjected to a variety of fixatives and staining reagents.

Though autopsy material is perhaps not the best source for obtaining normal tissue, it is considered satisfactory for this study. In view of the changes in the parathyroid gland which occur in nephritis (MacCallum [20] and Pappenheimer and Wilens [13]), the glands from nephritic patients were not included within the normal group nor were those from children with rickets. The glands used were from persons dying of a wide variety of diseases, but no histologic changes were present which could be associated with the morbid processes. The great majority of the autopsies were performed within three or four hours after death, and the glands, when compared with fresh surgical material, presented no postmortem change.

Fresh glands from autopsy were fixed in absolute alcohol, formaldehyde solution U. S. P. (1:10), alcohol plus formaldehyde solution, Regaud's fluid,[21] Zenker's solution, Meve's fluid,[22] Müller's fluid plus dilute solution of formaldehyde, Bouin's fluid, and acetic acid bichromate solution. The stains used consisted of hematoxylin and eosin, iron hematoxylin, Van Gieson's stain, Heidenhain's iron hematoxylin, neutral gentian, pyronine methyl-green, phosphotungstic acid hematoxylin, Best's carmine, Bensley's [23] (Altmann's aniline acid fuchsin) stain, sudan IV, nile blue, osmic acid, Smith-Dietrich stain [24] and a modification of Mallory's aniline blue stain. Other methods were also employed but gave no further detail than those named.

With a modification of Mallory's aniline blue stain, the granules of the parathyroid cells stood out clearly as bright cherry-red masses. This method makes possible the easy recognition of a single oxyphil cell in a whole section. The stain is noted here in the hope that it, or some further modification of it, may prove of value to those desiring a stain of this type.

A Modification of Mallory's Aniline Blue Stain.—The directions for the modification of Mallory's aniline blue stain are as follows:

1. Fix tissue in Zenker's solution, embed in paraffin, cut sections from 4 to 6 microns thick, carry down to water.
2. Stain very deeply with alum hematoxylin—from three to six minutes.
3. Blue well in lithium carbonate solution. Rinse in tap water.
4. Stain in aniline fuchsin (see next paragraph).
5. Wash quickly in distilled water.
6. Immerse in 1 per cent phosphomolybdic acid—ten minutes.
7. Transfer directly to modified aniline blue (see subsequent paragraph).
8. Wash in 95 per cent alcohol until clouds of blue stop.
9. Treat with absolute alcohol, then with xylene, and mount in balsam.

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20. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **16**:87, 1905.

21. Regaud's fluid is made up of 3 per cent potassium bichromate, 4 parts, and neutral formaldehyde, 1 part. The tissues must be kept in the dark and the fluid changed every day for four days. Mordant in 3 per cent potassium bichromate for eight days, changing fluid on the second, fourth and sixth days.

22. The formula for Meve's fluid is: chromic acid, 0.5 Gm.; 1 per cent sodium chloride, 15 cc.; 2 per cent osmic acid, 4 cc.; glacial acetic acid, 3 drops. Fix for from one to eight days.

23. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924.

24. Mallory and Wright,[23] p. 187.

The aniline fuchsin is made up as follows: Mallory's aniline water, 100 cc., and acid fuchsin, 20 Gm. This solution is not good after ten days. During the first two days the slides should be left in the stain for four minutes, and for from five to seven minutes from the third to the tenth day inclusive.

The aniline blue is made up as follows: aniline blue, 0.5 Gm.; orange G, 2 Gm., and distilled water, 100 cc. This solution keeps well.

CLASSIFICATION OF EPITHELIAL CELLS

On cursory examination the cells appear to show four distinct and separate forms, but, by detailed study and use of a variety of staining methods, it is possible to trace the transition of cells from one type to another. It is probable that these cells represent different phases of cellular activity and, though the classification presented here speaks of distinct cell types, it must be remembered that different stages of cytomorphosis are being described rather than clearcut groups of cells. Castleman and Mallory [25] favor the term "water clear" or, as Getzowa [26] named it, the *wasserhelle* cell. This variety of cell represents the ballooned or large vesiculated pale principal cell of my classification. The pale and dark principal cells, as described later, are separate and distinct phases of cytomorphosis. Since the *wasserhelle* cell represents but one of the phases of one of the more common varieties of cells, the pale principal, it is not considered to be of sufficient importance to merit a separate classification. The term *wasserhelle* is very descriptive, however, and is of value in describing cells associated with certain abnormalities of the parathyroid glands.

Welsh [27] first divided the parenchymal tissue into principal and oxyphil cells; later he divided the principal cells into four types. Erdheim [28] described two forms of the oxyphil cell: the large pale and the small dark. Marine's [14] classification consists of three varieties: the clear chief, the dark chief and the oxyphil. Cooper [17] described ordinary epithelial cells, empty cells and oxyphil cells, while Turnbull and his co-workers [19] spoke of light and dark oxyphil and principal cells. Castleman and Mallory [25] stated, "We have elected to recognize four major cell types and have been forced to admit the existence of transition types." The cell types noted by these authors are: normal chief or principal, *wasserhelle*, pale oxyphil and dark oxyphil. These are only a few of the classifications used, but it will be readily seen that there is need for a more uniform nomenclature. At the risk of further com-

25. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

26. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907.

27. Welsh, D. A.: J. Anat. & Physiol. **32**:380, 1897-1898.

28. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **33**:158, 1903.

plicating the issue, but with the desire to clarify it, the following classification is suggested:

Pale Principal Cells.—These cells (fig. 2), along with the dark type, form the bulk of the gland, are present throughout life and show a slight relative decrease in number with advancing age. In a 6 week embryo the parathyroid was entirely composed of these cells, while in a fetus of 6 months, though most of the cells were in this phase, a few of the dark variety were also present. Depending on extracellular pressure, the shape of these cells varies from round to cuboidal or polygonal. The size ranges from 7 to 15 microns, with the nucleus measuring from 5 to 6 microns. The cytoplasm consists of a meshwork of irregular particles of various sizes which are stained a light red by the pyronine methyl-green method. The cells show vesiculation, and as this process increases they become larger, the cytoplasmic network is pushed to the periphery of the structure, and there is an increased prominence of the limiting membrane. In the more extreme degrees of vesiculation the nucleus, which is usually eccentric, appears to be lying free in a large empty cell. The nucleus is a pale staining structure, irregularly round, and contains a variable number of darker staining chromatin granules of various sizes and shapes. The pale principal cells are essentially basophilic. With the modified Mallory stain the cytoplasm, when visible, is light brownish, with hematoxylin and eosin light bluish purple, and with pyronine methyl-green it is pale bluish green.

Dark Principal Cells.—This type of cell very closely resembles the pale variety described. The cells are present in infancy, and there is a relative increase in their number with advancing years. As will be discussed later, these cells appear to represent an intermediate stage between the pale principal and the oxyphil types. They are slightly smaller than the pale variety, with their greatest diameter measuring from 6 to 12 microns. The cytoplasm, when stained by the modified Mallory method, is light brownish with often a few cherry-red granules about the periphery of the cell. The cytoplasmic network is irregularly fragmented, and vesiculation is present. The nucleus is usually smaller than in the pale type and measures from 3.5 to 5 microns, and its chromatin granules are closely packed. It is somewhat irregular in shape and takes a deep stain in contrast to the nucleus of the pale type.

Dark Oxyphil Cells.—The cells described under this heading appear to be an intermediate stage between the dark principal and pale oxyphil types. They are normally relatively few and may represent a comparatively rapid state in cell transition. They measure from about 6 to 14 microns, the average being from 7 to 9 microns in diameter. They are usually polygonal, and the cytoplasm is almost or entirely filled by granules of a fairly uniform size, which when stained by the modified Mallory

method stand out prominently as bright cherry-red masses (fig. 3). The cell membrane is definite and sharply delimits the cell. The cytoplasm is stained deep pink by hematoxylin and eosin, while pyronine methyl-green gives a deep green color. Examination under high magnification often reveals a few scattered vesicles of various sizes. The nucleus measures from 4 to 5 microns in diameter, and is very similar in appearance to the nucleus of the dark principal cell.

Pale Oxyphil Cells.—These cells are much more numerous than the dark oxyphil type and are usually large and irregularly polygonal. Here

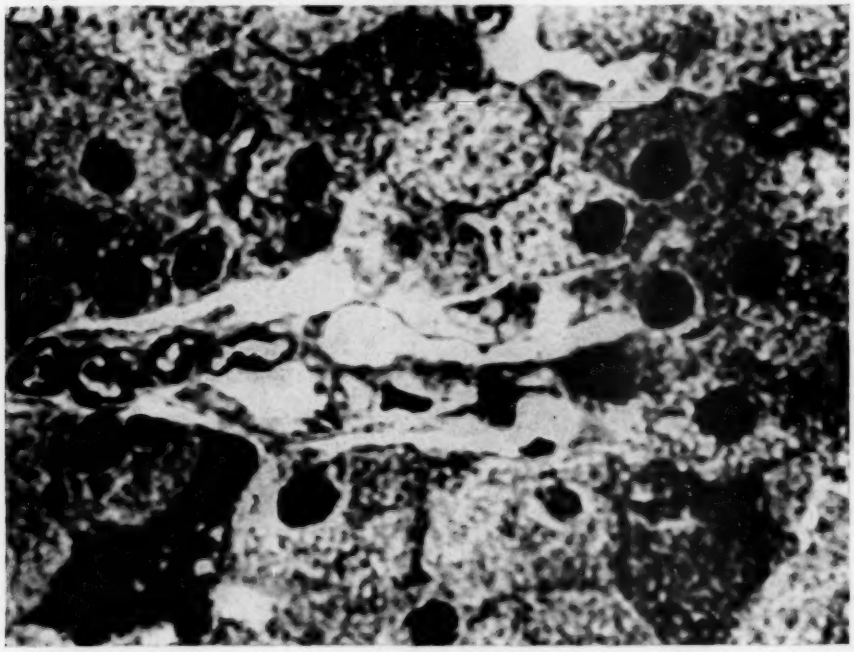

Fig. 3.—Pale and dark oxyphil cells; × 1,500.

as in other parathyroid cells the shape is influenced by pressure of adjacent cells, and almost perfectly round oxyphil cells have been noted entirely surrounded by fat. The pale oxyphils vary in diameter from 9 to 19 microns, the average being from 13 to 15 microns. Though, because of the relatively large amount of cytoplasm and small nucleus, thin sections for microscopic study show masses of oxyphilic tissue containing no nuclei, examination of serial sections reveals the fact that each oxyphil cell possesses a well defined nucleus. The cytoplasm stains reddish pink with hematoxylin and eosin, and by the pyronine methyl-green method the staining is lighter than in the dark type. The modified Mallory stain shows the cytoplasm to contain many cherry-red granular

masses. In some cases the granules almost completely fill the cytoplasm while in others, chiefly the larger ones, there is a varying amount of vesiculation. This vesiculation usually begins toward the central portion of the cell and often immediately adjacent to the nucleus. As vesiculation progresses these clear spaces appear to coalesce so that the nucleus seems to be floating freely in or about the center of the cell while the granules are concentrated in the periphery. The nucleus measures from about 4 to 6 microns, is paler than the dark type, has a homogeneous appearance and is often situated toward one side of the cell. Phosphotungstic acid hematoxylin differentiates well the nuclei of the oxyphil cells. The pale type stain a homogeneous color with a few fine irregular

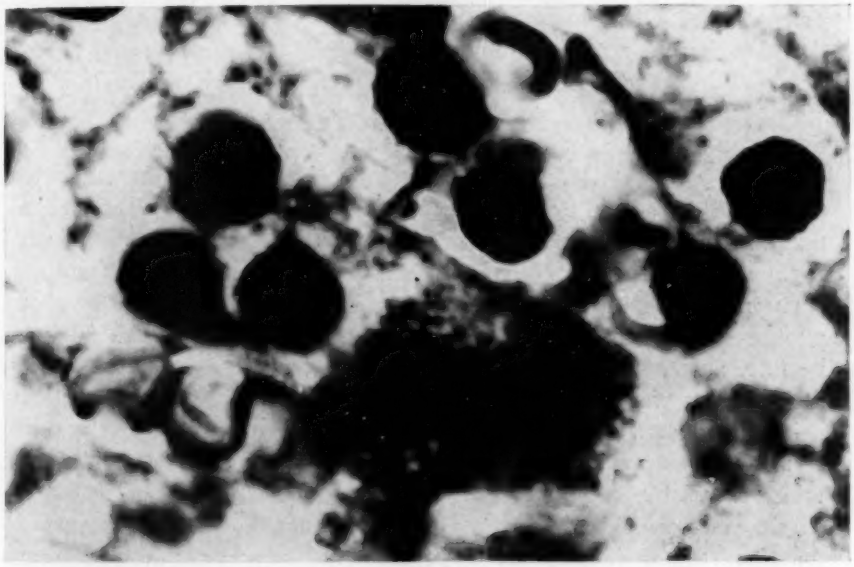

Fig. 4.—An oxyphil cell in the parathyroid of a child 2½ years old; × 2,350.

chromatin granules while the nuclei of the dark type are almost solidly packed with dark blue granules. Various intermediate forms are also seen, but most nuclei tend to be of the pale type.

Various authors report the oxyphil cells as being first found at different ages, but the youngest age at which they are usually noted is that recorded by Marine,[14] who stated, "The oxyphil cells usually begin to appear in late childhood (as early as the seventh year)." By careful examination of many sections stained by a variety of methods, including the modification of Mallory's aniline blue, I found oxyphil cells in the parathyroid glands of children as young as 7 years, 2½ years (fig. 4) and even 6 months (one instance). In the latter case these cells were

quite numerous, and a detailed account is noted later in this paper. In the parathyroid tissue of the other children only an occasional oxyphil cell was found, but those found were similar in every respect to those observed in the adult gland. The oxyphil cells increase in number with advancing age and thus serve in a rough way to allow an estimation of the age of a person.

Glycogen is found in every type of cell in the parathyroid gland. The substance is present in greater quantity in the principal cells than in other types but is also moderately abundant in the oxyphils. The last observation is not in accord with Castleman and Mallory [25] and Turnbull [19] but agrees with the work of Marine.[14] The latter has stated, "The glycogen content appears to be independent of age, sex and disease . . ." My findings concur with this statement with the exception that there is a marked diminution in the glycogen content of glands obtained from patients with diabetes. Further it is found in common with Marine that the glycogen content is not lost as quickly as in other tissues and may be demonstrated as long as sixteen hours after death.

Because of the difficulty in securing perfectly fresh human parathyroid glands only a few mitochondrial stains were done. It was noted that all forms of cells contained these small cytoplasmic elements and that they were much more numerous in the principal cell types. The most successful method was that of fixation in Meve's fluid followed by phosphotungstic acid staining. This procedure revealed the mitochondria to be fine, short, rodlike, deep purplish-blue structures. Several microincinerations were carried out at a temperature of 550 F., the sections being incinerated from two to five hours. The cytoplasm of the principal cells was practically free from ash while that of the oxyphils contained a variable amount of finely divided particles.

Several formaldehyde-fixed glands were cut on the freezing microtome and the sections stained for fat by the sudan IV, nile blue, osmic acid and Smith Dietrich methods. Fat was found in all types of cells, but it was in larger droplets and greater quantities in the principal cell forms than in the oxyphils. The mature oxyphil cell often contained near the nucleus a clear, round vacuolated space which by suitable staining methods was shown to be a fat globule. Some granular yellowish-orange intracellular material occasionally seen in hematoxylin and eosin-stained sections was proved to consist of a lipochrome substance. Polarized light revealed only an occasional doubly refractile crystal within the principal cells. The adipose tissue of the parathyroid was irregularly distributed as single cells or groups of cells which tended to be more prominent in the periphery and poles of the gland.

Cooper [17] has stated, "Eosinophil leucocytes are very numerous at puberty, but are in their greatest numbers in adult life, when they are

present chiefly in the blood vessels and in the connective tissue capsule trabeculae, though infrequently they occur in small intercellular spaces." I have been unable to confirm this statement for, though eosinophils were found, their number was not in excess of normal.

Tissue mast cells, though rare in the parathyroid glands of children, become prominent about puberty and early adult life. They contain many rounded, deep purplish-blue granules and are usually found in the connective tissue strands but may occasionally be seen in small spaces between the parenchymal cells.

CELL ARRANGEMENT

The cells frequently appear in rows or columns which are folded on themselves. With an increase in stroma they are often divided off into irregular masses or clumps and in some cases assume an alveolar appearance. Acini are found in glands of all ages including those of infants, Cooper [17] reporting these structures in a child of 2 months. The cells lining the alveoli are usually low columnar or cuboidal and generally are the pale or dark principal cells. From time to time one or more dark or pale oxyphil cells may be seen helping to form the acinar wall. Alveolar structures are also noted in which the lining cells are all oxyphilic; in these the lumen is small. The number of acini and their position within the gland are extremely variable. They usually contain a clear colloid material which stains evenly with eosin. All acini have been examined carefully, but all cells showed intact cell membranes and presented no appreciable degenerative changes. These observations indicate that the colloid material, when present, is a secretory product and not the result of cellular disintegration.

Any one type of cell may be found lying beside any other type. There also appears to be no definite arrangement of cells in relation to the blood supply. Though oxyphil cells tend to appear in groups near the poles and periphery of the gland, an examination of these portions reveals that they possess an excellent vascular network of thin-walled sinusoidal capillaries.

CELL TRANSITION

As noted, I am of the opinion that there occurs in the parathyroid a definite transition of cells from one type to another. This hypothesis has been mentioned and supported by Turnbull,[19] Marine [14] and others, and evidence in favor of this concept is becoming increasingly stronger.

Pale principal cells appear to be the primary cells of the parathyroid for they form the bulk of the parenchyma of every normal gland. In many of the glandules from young children the principal cells, chiefly the pale variety, are the only ones seen, while in the cat, rat and dog these are the only cells present throughout life. In the 6 week embryo

the cells are all of the pale principal variety (fig. 5). One finds a relative decrease in the number of pale principal cells and an increase in that of the dark species as older parathyroids are examined. The dark cells are seen immediately adjacent to the pale ones and have every appearance of being derived from the latter. The glands of infants and children usually show only an occasional oxyphil cell. In the parathyroid of a baby 2 years of age only three widely separated oxyphil cells are found. These cells are identical in every respect with the dark oxyphil type occasionally seen in the glands of older persons. They have no deficiency of blood supply and are surrounded by perfectly normal pale and dark principal cells. Morphologically they appear to be derived

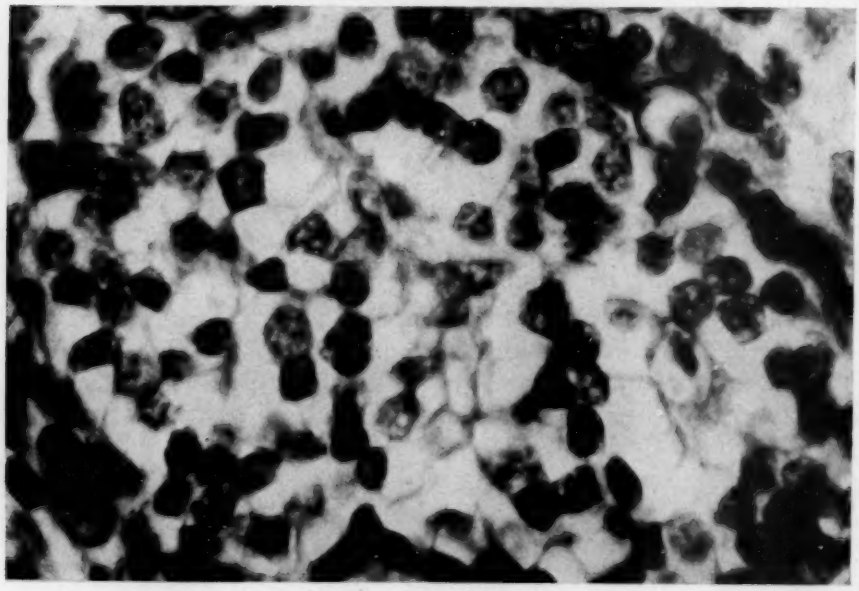

Fig. 5.—Principal cells in the parathyroid gland of a 6 week embryo; × 900.

from the dark principal cell type by the simple addition of red granules to the cytoplasm. A further point in favor of this transition is the not infrequent finding of dark principal cells with just a few granules in the cytoplasm which cannot be definitely classified with the oxyphil series. There is a marked increase in the number of oxyphil cells in the older glands. This increase is accompanied by an increase in adipose content and a decrease in the total number of parenchymal cells. All evidence indicates that the oxyphil cells must be derived from preexisting cells, i. e., the principal type. The formation of the pale oxyphil from the dark oxyphil series is brought about by an increase in size and in the granular content of the cytoplasm and a change in the nucleus so that

it is less deeply stained. The results of examination of sections stained with pyronine methyl-green agree with those of Turnbull,[19] who stated: "Further the basophil cytoplasmic net of the principal cells usually contains more deeply staining basophil particles whilst similar particles sometimes lie upon the basophil membrane of pale oxyphil cells and occasionally lie isolated within the oxyphil cytoplasm. The appearances suggest that the oxyphil cells are principal cells in which the cytoplasm has been so charged with oxyphil granules that the basophil net has been more or less reduced to a limiting membrane."

I am quite unable to present proof of the physiologic significance of the different cells. The correlation of the histologic appearance and the physiologic function of the parathyroid in different conditions of age, health and disease is not attempted but presents an interesting problem for discussion.

The principal cells are usually the only variety present in the glands of infants and young children, yet since this is the period during which bone formation is most active it is concluded that these cells are capable of the secretory activity concerned in the rôle of the parathyroid gland in calcium metabolism. The functional activity of the oxyphil cells cannot be determined as yet. It is significant, however, that oxyphil cell tumors in patients presenting the characteristic clinical picture of hyperparathyroidism have been reported by Hunter and Turnbull,[19] Gutman and his co-workers,[29] Warren and Morgan [30] and others. My own findings agree with those of the other workers and hence it is concluded that in some cases at least oxyphil cells are capable of producing either an excessive or an abnormal parathyroid secretion. The relief of symptoms and return to normal calcium metabolism after operative removal are proof that the oxyphil cell tumor was the factor causing the hyperparathyroidism. Castleman and Mallory [25] and others have reported parathyroid tumors consisting of various transitional types of cells. It is evident that cells in various phases of transition are capable of producing tumors, and the secretion from any of these different varieties of cells will, if in excess of normal, produce hyperparathyroidism.

Another point of interest is the relationship of cellular glycogen and fat content. As noted earlier, the principal cell forms contain the largest amounts of both these substances while little is present in the mature oxyphil forms. The direct relationship between the amounts of intracellular glycogen and fat appears to indicate that these substances are in some way concerned with the functional activity of these cells. This seems to lend further weight to the claim that the oxyphil forms possess

29. Gutman, A. B.; Swenson, P. C., and Parsons, W. B.: J. A. M. A. **103**:87, 1934.

30. Warren, S., and Morgan, J. R. E.: Arch. Path. **20**:823 (Dec.) 1935.

a small amount of functional activity. The single mitotic figure found in all the sections examined occurred in an oxyphil cell. This observation is still further proof that the oxyphil forms are capable of cellular activity and are not merely inert structures.

The study of some of the pathologic processes occurring in the parathyroid suggests the possibility that the described cytomorphologic changes may be reversible. The presence of fat cells without an increase in size of the gland and with a definite decrease in the number of parenchymal cells is positive proof of fat replacement. Large pale oxyphil cells are occasionally noted which contain fat droplets, have a degenerated appearance and are considered to represent the end stage of the cell life. These cells are the only type which show fragmentation or evidence of degenerative change.

A parathyroid gland from a 6 month old infant, who died of toxic myocarditis, presents all stages of cell transition. The unknown factor which produces these morphologic changes had been active even to the extent of causing fat replacement. Most of the cells are of the light or dark principal cell type. All stages from dark principal cells with a few granules to dark oxyphil cells with the cytoplasm fully charged with cherry-red granules are present. The change from dark to light oxyphil types is easily followed and evidence of fat replacement readily discerned. All the cells of the gland correspond exactly with their adult counterparts, but in this case the speeding-up of the process of transition permitted the definite recognition of all types within one section.

Further conclusive evidence of this transition is obtained by examination of parathyroid tumors obtained in cases of hyperparathyroidism. Some of the tissue used in this study contains cells which correspond exactly to each of the described types of cells. There are also present all intermediate stages of cytomorphosis, and the morphologic variations between the different phases of cellular types are readily discernible. The results of this investigation present the conclusion that the pale principal cell is the primary type and that from it all other forms develop.

SUMMARY

A study of more than three hundred normal parathyroid glands, obtained from persons varying in age from a 6 week fetus to adults of 70 years, is reported. The work presents a norm from which pathologic variations may be determined. There is one primary cell, the pale principal, from which all other forms develop. A gross and microscopic description of the glands is presented along with evidence in support of cell transition and functional activity of each cellular form. A new staining technic for granules, a modification of Mallory's aniline blue stain, is described.

MYASTHENIA GRAVIS

A STUDY OF POSTMORTEM OBSERVATIONS, INCLUDING THE DEMONSTRATION OF GRAM-POSITIVE BACTERIA (STREPTOCOCCI) IN AND BETWEEN THE MUSCLE FIBERS

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ROCHESTER, MINN.

Necropsy was performed in a case of myasthenia gravis shortly after a similar examination had been made in a case of dermatomyositis, and microscopic study of the muscles in the two cases revealed certain resemblances. The collections of lymphocytes in the muscles in both cases suggested a chronic infection. The inference was natural that the underlying cause of myasthenia gravis was a type of infection which became localized in the muscles in a manner similar to the localization of rheumatism in joints and also that since the lesions in myasthenia gravis, scleroderma and dermatomyositis often present similar pictures, they might all be related to a common type of infection with different localizing properties. This observation led to the present study of the pathologic changes in all the cases of myasthenia gravis in which necropsy had been performed.

Many investigators have noted the relationship between myasthenia gravis and infection, especially of the upper part of the respiratory tract. Remak [1] described a case in which myasthenia gravis followed an attack of influenza; Jolly [2] reported a case in which there had been a preceding attack of otitis media, and von Kétly [3] collected one hundred and thirty-four cases and found that the condition had followed an infectious disease in twenty-six of them. Hall [4] reported a case in which encephalitis had preceded myasthenia gravis; Stiefel,[5] an instance in which the disease had followed an attack of acute articular rheumatism, and Campbell and Bramwell,[6] a case in which myasthenia gravis had developed after scarlet fever. Boothby [7] found that in seven of twenty cases (35

This study was undertaken at the suggestion of Dr. Walter M. Boothby, and the work was done in the Section on Pathologic Anatomy at the Mayo Clinic under the direction of Dr. H. E. Robertson.

1. Remak, E.: Arch. f. Psychiat. **23**:919, 1892.
2. Jolly, F.: Berl. klin. Wchnschr. **32**:1, 1895.
3. von Kétly, Ladislaus: Deutsche Ztschr. f. Nervenh. **31**:241, 1906.
4. Hall, G. W.: Arch. Neurol. & Psychiat. **17**:561, 1927.
5. Stiefel, Fritz: Schweiz. med. Wchnschr. **52**:1056, 1922.
6. Campbell, Harry, and Bramwell, Edwin: Brain **23**:277, 1900.
7. Boothby, W. M.: Proc. Staff Meet., Mayo Clin. **7**:557, 1932.

per cent) in his series the patient gave a history of acute infection, which had occurred shortly before the onset of the disease. In two cases (28.5 per cent) in my series in which postmortem examination was made, infection had occurred a short time before the onset of the typical symptoms of myasthenia. Three patients (42.8 per cent) had had infections of various types, usually of the upper part of the respiratory tract, which apparently had produced an exacerbation of the disease that resulted in death.

Jotezko [8] suggested that in myasthenia gravis one is dealing with exhaustion or liability to exhaustion on the part of the nerve endings in the muscles. Campbell and Bramwell [6] suggested, without direct evidence, that "in myasthenia gravis, a toxin, probably of microbic origin, circulates in the blood and acts selectively on the lower motor neuron so as to modify its functional activity." It is probable that in this disease the weakness is due to toxins of some kind and that the weakness may persist intermittently for a long time. Often the condition terminates in death; however, no grossly discoverable lesions necessarily are noted. Patrzek [9] reported a case of myasthenia gravis in which the disease had been preceded for two years by scleroderma of the face, hands and feet. Brock [10] concluded that dermatomyositis and diffuse scleroderma are probably of infectious origin. Furthermore, it is significant that the course of myasthenia gravis, with its exacerbations and remissions, often resembles that of an infection.

Just where a toxin acts in myasthenia gravis is not known. Keschner and Strauss [11] expressed the opinion that it is at the periterminal network. Dale and Feldberg [12] concluded that the weakness is the result of disturbance in the myoneural junction. Pritchard,[13] in a study of the effect of the di-methylcarbonic ester of m-oxyphenyl-tri-methylammonium sulfate in cases of myasthenia gravis, suggested that the site of action of the drug is the same as that of the pathologic process causing the weakness. This site he located at the place of the normal transmission of excitation from the somatic nerve endings to the voluntary muscle fibers. Since the symptoms were improved by the administration of amino-acetic acid, Boothby [7] and others have suggested that the characteristic fatigability might be the result of interference with the reformation of phosphocreatine, which is thought to play an important

8. Jotezko, quoted by Buzzard.[20]

9. Patrzek, F., cited by Keschner and Strauss.[11]

10. Brock, W. G.: Arch. Dermat. & Syph. **30**:227, 1934.

11. Keschner, Moses, and Strauss, Israel: Arch. Neurol. & Psychiat. **17**:337, 1927.

12. Dale, H. H., and Feldberg, W., cited by Pritchard.[13]

13. Pritchard, E. A. B.: Lancet **1**:432, 1935.

part in muscular contraction. Nevin,[14] however, in a study of the chemical action of muscles in myasthenia gravis, found that activity and recovery in the myasthenic muscles differ in no significant way from similar processes which are characteristic of healthy muscles.

Wilks [15] was the first to record the observations at necropsy in a case of myasthenia gravis, but he was unable to demonstrate any abnormal anatomic changes. Mayer,[16] using the Marchi stain, demonstrated strips and flakes of degenerated myelin in the intramedullary portion of the cells of the anterior horn and the hypoglossal nerve roots. Jolly,[2] in a study of the disease, observed nothing abnormal in either the nervous or muscular system. He reported the first case in which the muscles from the body of a patient who had died of myasthenia gravis were examined microscopically. Widal and Marinesco [17] observed chromatolysis in the nerve cells of the third, sixth, seventh and twelfth nuclei. Degenerated fibers were seen in the roots of the third, seventh and twelfth nerves.

Sossedorf,[18] according to Buzzard, was the first to describe collections of lymphocytes in the muscles of a patient who had myasthenia gravis. Weigert [19] reported a case in which he observed numerous foci of lymphoid cells in the muscles; a few "epithelial" cells were scattered throughout these foci. These foci were observed in the deltoid muscle, the diaphragm, the myocardium and the epicardium. He also noted a malignant tumor of the thymus in the same case, and he considered these collections of cells to be metastatic. Buzzard [20] made a thorough study of material taken post mortem in five cases of myasthenia gravis. He called the foci of lymphocytes "lymphorrhagias." Buzzard noted collections of lymphocytes in varying numbers in some of the muscles in every case in his series. In several instances he observed foci in the heart, the liver and the adrenal and thyroid glands. He also observed an early stage of muscular degeneration in every case. Marburg [21] demonstrated lipoid droplets in the muscle fibers in a case of myasthenia gravis. Knoblauch,[22] Csíky [23] and others called attention to the great variability in the staining of the sarcoplasm.

Buzzard, in a study of nerve tissues, observed a few collections of lymphocytes in the substance of several posterior root ganglions.

14. Nevin, S.: Brain **47**:239, 1934.
15. Wilks, Samuel: Guy's Hosp. Rep. **23**:54, 1877.
16. Mayer, C.: Neurol. Centralbl. **13**:126, 1894.
17. Widal, and Marinesco, G., quoted by Campbell and Bramwell.[6]
18. Sossedorf, Marie, cited by Keschner and Strauss.[11]
19. Weigert, Carl: Neurol. Centralbl. **20**:597, 1901.
20. Buzzard, E. F.: Brain **28**:438, 1905.
21. Marburg, O.: Ztschr. f. Heilk. **28**:111, 1907.
22. Knoblauch, A.: Frankfurt. Ztschr. f. Path. **2**:57, 1908.
23. Csíky, Josef: Deutsche Ztschr. f. Nervenh. **37**:175, 1909.

Mandlebaum and Celler [24] described lymphocytic foci in the perivascular lymph spaces of several small capillaries in the gray matter near the nucleus of the vagus nerve. Mott and Barrada [25] at necropsy observed throughout the central nervous system a general diminution of the basophilic substance; this was most marked in the small and large cells of the thalamus. McAlpine [26] reported a case in which there were inflammatory changes and "mucocytic degeneration" of the spinal cord.

Skinner,[27] after study of a section of muscle from the leg of a patient who had died of myasthenia gravis, made the following statement: "There is a marked increase in the number of sarcolemma nuclei. These nuclei are seen between the muscle fibers in much greater profusion than normal."

Oppenheim [28] was the first to report "enlargement" of the thymus in cases of myasthenia gravis. Burr and McCarthy [29] reported a case in which there was an abscess of the thymus; in the same year, von Laquer and Weigert [30] described a case in which a lymphosarcoma of the thymus had metastasized to the deltoid muscle, diaphragm, heart and pericardium. Palmer [31] collected thirty-four reports of necropsies from the literature. In ten of these cases there was involvement of the thymus, and in seven of the ten metastatic deposits were present in the muscles. According to Keschner and Strauss, enlargement of the thymus occurs in about one half of all the cases of myasthenia gravis. Bell [32] expressed the opinion that the thymic tumors which occur in cases of myasthenia gravis form a distinct group, unlike any other tumors of the thymus.

EXAMINATION OF MATERIAL TAKEN AT AUTOPSY

My study is based on the results of pathologic examination in seven cases of myasthenia gravis in which necropsy was performed at this clinic. Five of these cases were encountered in the past two years.

Collections of lymphocytes were noted in some of the sections of muscle in five of the seven cases. These collections (fig. 1 *a*), which varied in size, were observed in the tissue spaces between the muscle fibers. They were more easily identified in the sections in which the muscle fibers were cut transversely than in those in which the muscle fibers were cut in other planes. They did not appear

24. Mandlebaum, F. S., and Celler, H. L.: Proc. New York Path. Soc. **8**:88, 1908.
25. Mott, F. W., and Barrada, Y. A.: Brain **46**:237, 1923.
26. McAlpine, Douglas: Brain **52**:6, 1929.
27. Skinner, E. F.: J. Neurol. & Psychopath. **4**:344, 1924.
28. Oppenheim, H., quoted by Skinner.[27]
29. Burr and McCarthy, quoted by Palmer.[31]
30. von Laquer, Leopold, and Weigert, Carl: Neurol. Centralbl. **20**:594, 1901.
31. Palmer, A. S. M.: Guy's Hosp. Rep. **62**:55, 1908.
32. Bell, E. T.: J. Nerv. & Ment. Dis. **45**:130, 1917.

in any particular group of muscles, except in the last two cases, in which greater involvement of the muscles of the pharynx was observed. In one case, a lymphocytic focus was also noted in the muscle of the gastric wall.

While many muscle fibers appeared to be normal, atrophy was observed not only in areas adjacent to the collections of lymphocytes but also in fields free from these foci. A form of degeneration (fig. 1 *b*) was seen in some of the sections of muscle in all the seven cases.

The nerve and brain tissues in these cases revealed no unusual changes.[33]

In two of the seven cases thymomas were observed.

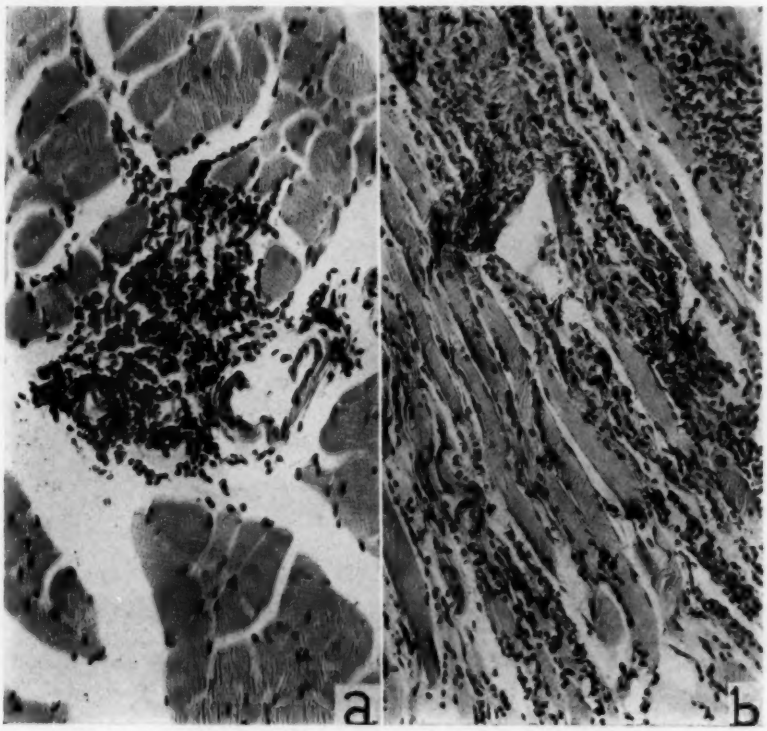

Fig. 1.—Photomicrographs of sections of muscle in a case of myasthenia gravis. A section of the diaphragm showing a focus of lymphocytes is shown in *a* (× 185); *b* is a section of the crico-arytenoid muscle, showing marked degeneration of the muscle fibers and collections of lymphocytes (× 115). Hematoxylin and eosin stain.

Bacteriologic observations in cases of myasthenia gravis by Rosenow and Heilman,[34] to be reported later, prompted examination of the tissues for the presence of bacteria in these instances. Sections were stained by the Rosenow and Sanford modification [35] of the Gram-Weigert method, and bacteria were noted

33. I am indebted to Dr. J. W. Kernohan for examination of the nerve tissues.
34. Rosenow, E. C., and Heilman: Personal communication to the author.
35. Rosenow, E. C., and Sanford, A. H.: J. Infect. Dis. **17**:219, 1915.

in some of the sections in each case (figs. 2*a* and *b* and 3*a*). The organisms were gram-positive diplococci, possibly streptococci. Occasionally, they appeared swollen and round; less often, they assumed an elongated, swollen bacillary form (fig. 3*b*). Some organisms were seen in small groups; others were scattered throughout the section. In no section were large numbers of organisms observed; on the contrary, they were at times discovered only after diligent search. They were situated in and between the muscle fibers. The bacteria were never seen in the lymphocytic foci, yet occasionally they appeared at the periphery of these foci. Examination of the sections from a few organs (liver, adrenal gland, thymus and kidney) by this method did not reveal the presence of these cocci or of other bacteria.

In one case sections of muscle were taken under sterile precautions an hour and a half after the death of the patient and before the body was embalmed. Sections were also taken with the usual procedure after the body was embalmed.

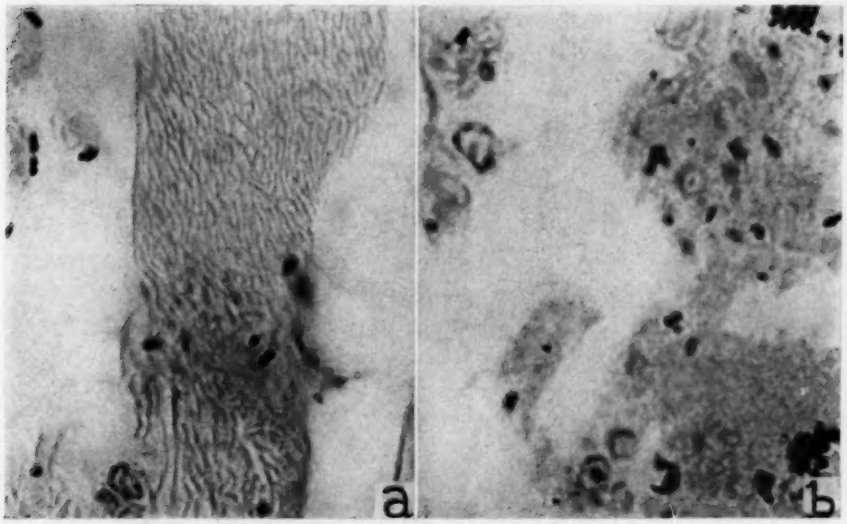

Fig. 2.—Photomicrographs of sections of muscle in a case of myasthenia gravis. A section of the pectoral muscle is seen in *a* and in *b* a section of the left pharyngeal muscle, showing diplococci. Rosenow modification of the Gram-Weigert stain; × 1,000.

In both groups of sections the organisms were noted in approximately the same numbers. Examination of the corresponding muscles in seven cases in which myasthenia gravis was not present served as a control. A search which was much more diligent than that employed in the cases of myasthenia gravis revealed no bacteria in or between the muscle fibers in sections in the cases used as controls. The diagnoses in these cases were as follows: (1) neurofibroma of the left eighth nerve and basilar meningitis, (2) coronary sclerosis with infarction of the heart, (3) spongioblastoma of the brain and bronchopneumonia, (4) chronic infectious arthritis with fatal pulmonary embolism, (5) hypertension with cerebral arteriosclerosis and infarction of the brain, (6) sarcoma in the left part of the

chest and postoperative shock and (7) squamous cell carcinoma of the vulva and chronic infarction of the heart.

The following measures were taken in an attempt to exclude the factor of artefact or contamination: The materials used in staining, including the fixatives, were examined by means of cultures and stained preparations and were observed to be free from streptococci, although contaminants from the air, hay bacilli and so forth were noted. Several sections of muscle were dipped in cultures of streptococci before being fixed in a solution of formaldehyde. On section, bacteria could be observed in the connective tissue surrounding the muscle, but no organisms were seen in or between the muscle fibers. A few sections of muscle were cut with instruments which purposely were grossly infected, and an occasional organism could be noted around the periphery of the section but none were observed in or between the muscle fibers.

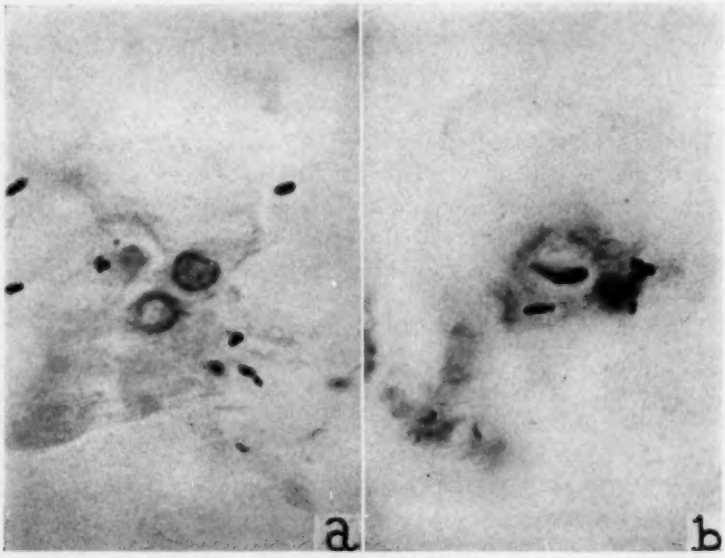

Fig. 3.—Photomicrographs of sections of muscle in a case of myasthenia gravis. A section of the crico-arytenoid muscle, showing diplococci, is seen in *a*, and in *b*, a section of muscle showing a swollen bacillary form of the diplococcus. To the right of this organism is a small diplococcus of fairly normal size and above this another swollen form which is not quite in focus. Rosenow modification of the Gram-Weigert stain; × 1,000.

COMMENT

Many pathologists entertain the belief that bacteria are frequently to be observed in all tissues, particularly after death, but the literature does not support the conclusion that muscle is frequently contaminated. An excellent review of the older papers bearing on the subject is that of Ford,[36] while among the recent reports are those of Burn[37] and

36. Ford, W. W., quoted by Bastian, H. C.: Lancet **2**:108, 1904.
37. Burn, C. G.: J. Infect. Dis. **54**:388, 1934; **54**:395, 1934.

Gulbrandsen.[38] In muscles from the bodies of seven patients who died of causes other than myasthenia gravis, no bacteria could be observed. In muscles from seven patients with myasthenia gravis, gram-positive bacteria resembling streptococci were noted in every case. The possibility of contamination of the tissues by the microtome knife or by fluids used in preparation was excluded.

SUMMARY AND CONCLUSIONS

Collections of lymphocytes were observed in all but two of seven cases of myasthenia gravis. In all the cases there was a form of degeneration in some of the sections of muscle. Thymomas were observed in two cases. Gram-positive diplococci and ovoid cocci which sometimes resembled bacillary forms were noted within or between the muscle fibers in some of the sections of muscle in all seven cases of myasthenia gravis. In seven control cases no organisms could be demonstrated in these positions. The possibility of artefact or contamination was excluded. The fact that gram-positive organisms were observed in the muscles of all the patients studied who had myasthenia gravis and were not seen in the muscles of a similar number of control subjects suggests that these bacteria may be the origin of the toxin which produces the characteristic fatigability in this disease.

38. Gulbrandsen, Lars: Proc. Inst. Med. Chicago **10**:250, 1935.

RÔLE OF THE PIA IN ENCEPHALOMENINGITIS AND MENINGO-ENCEPHALITIS

H. W. WILLIAMS, M.D.
SCHENECTADY, N. Y.

Spielmeyer [1] regarded poliomyelitis as encephalomeningitis. According to his views, the meningeal changes are secondary to those in the nerve tissue. Poliomyelitis, in other words, stands in contrast to certain forms of meningo-encephalitis in which meningitis is the disease and the involvement of the nerve tissue is a complication, a result of progression into the central substance. Before one enlarges on this concept of encephalomeningitis there is point in summarizing the observations that form the basis of Spielmeyer's opinion.

1. Acute, incompletely developed early lesions may exist in the cerebral cortex in cases of poliomyelitis without the overlying meninges evidencing any involvement.

2. "Symptomatic inflammation," such as was described by Spielmeyer [2] in septic encephalitic processes and the like and by Yü-Lin,[3] is also distinguishable in poliomyelitis. Figure 1, taken from the study of Spielmeyer, illustrates the condition. Within the focus there is a diffuse cellular infiltration consisting almost exclusively of leukocytes. The glia is markedly progressive. The blood vessels are inconspicuous. About the periphery of the focus, however, the blood vessels are prominent. The adventitia shows dense lymphocytic infiltration. The difference between the reaction pattern of the tissues about a focus and that within the lesion is dependent on a factor of position rather than of time. The peripheral reaction is an integral element of such foci. This is borne out by analogy with lesions of noninfectious diseases. In instances of softening of the brain and subacute combined degeneration in which the involvement of the nerve tissue is regarded without question as primary, the blood vessels about the periphery of the lesion may show a response of the lymphocytes and plasma cells, while within the focus there is no such reaction. This phenomenon is an integral part of such focal lesions, but it may be manifest for variable distances along the course of the blood vessels. In the vessels passing to or from the meninges, the infiltration may end at a point beyond the limits of

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From the Hirnpathologische Institut der deutschen Forschungsanstalt für Psychiatrie, Munich, Germany.

1. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **142**:159, 1932.
2. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **123**:161, 1930.
3. Yü-lin, Ch'Eng: Arch. Neurol. & Psychiat. **31**:1247, 1934.

the focus; it may persist until the vessel leaves or enters the meninges, or, finally, it may be seen in the meninges at one or both sides of the vessels for a variable distance. That is, there is a close relationship between the situation of the patchy meningitis, such as is seen in cases of poliomyelitis and analogous noninfectious diseases, and the location of the entrance or exit of the infiltrated vessels. Since the infiltration of the vessels is secondary, as already indicated, it follows that the meningitis is not primary but an aftermath of the involvement of the nerve tissue. One is dealing, therefore, with encephalomeningitis.

3. Superficial foci, such as those occasionally seen in the molecular layer of the cerebellum, produce a symptomatic inflammatory reaction

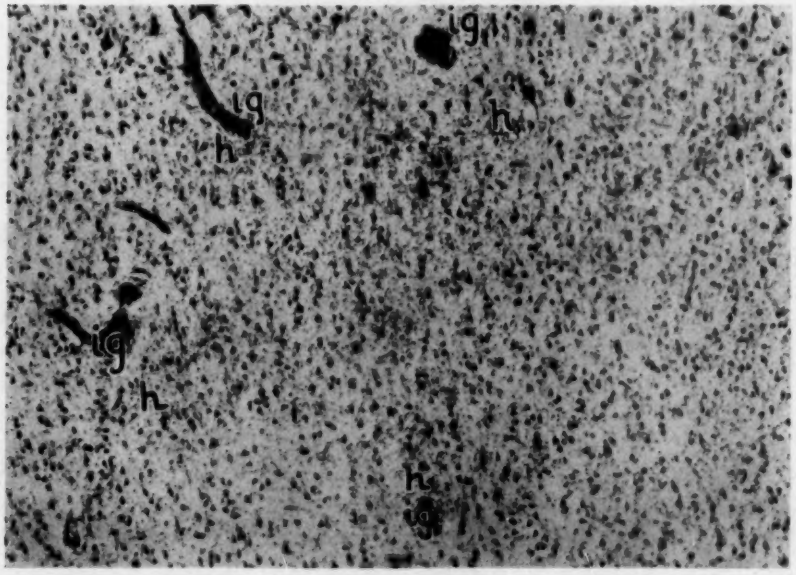

Fig. 1.—Focus in the motor cortex in a case of poliomyelitis. The area outlined by *h* approximates the limits of the focus, which consists principally of polymorphonuclear leukocytes and also of an exuberance of glia cells. About the periphery of the focus the walls of the veins are densely infiltrated with lymphocytes (*ig.*). Nissl stain (from Spielmeyer [1]).

localized in the overlying meninges in a fashion identical with that just described about the blood vessels.

To the aforementioned morphologic indications of encephalomeningitis may be added another. In poliomyelitis the cellular infiltration in the meninges is confined to the pia, that component of the meninges that lies in juxtaposition to the nerve tissue (fig. 2). The other components of the meninges—the subarachnoid space and the arachnoid—are essentially uninvolved. Experimental poliomyelitis of monkeys presents an identical picture.

Confirmation of this phenomenon as an indication of encephalomeningitis was looked for in other disease processes suspected of falling into this category. Borna disease in horses is often classified with poliomyelitis.[4] In this disease also the pia is seemingly the constituent of the meninges that is essentially involved. In figure 3*A* are shown the limitation of the infiltrating cells to the pia and the relationship of the pial infiltration to the vessels entering and leaving the nerve tissue, as Spielmeyer has reported in cases of poliomyelitis. The section was taken from the crest of a cerebral gyrus, where the elements of the meninges are more readily separated than in the sulci.

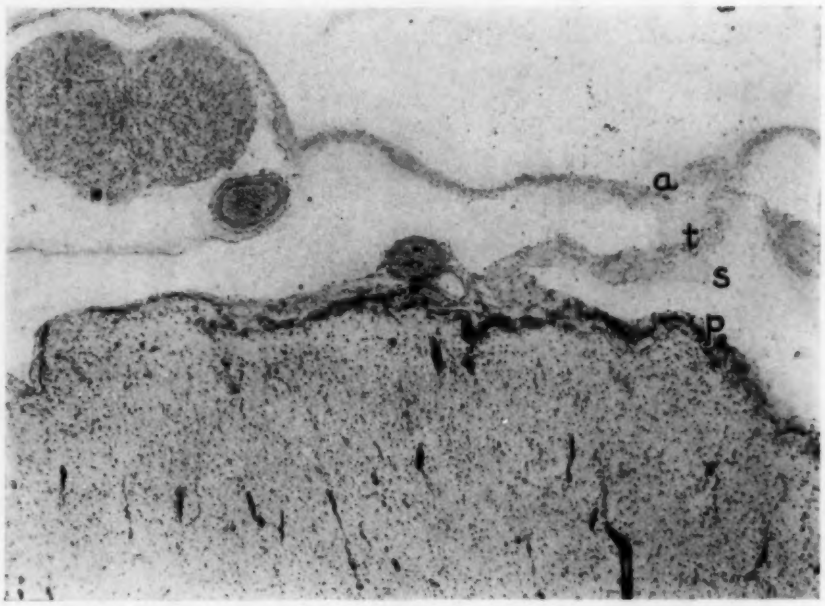

Fig. 2.—Section of the spinal cord in a case of poliomyelitis. The cellular infiltration is limited to the pia (*p*). The arachnoid (*a*), the subarachnoid space (*s*) and the trabecula of the arachnoid (*t*) are free.

Meningitis in acute epidemic encephalitis and rabies may be suspected of being restricted to the pia. It is true that the pia is involved and that the observations of Spielmeyer may be repeated in these diseases. However, scarcity of material in which the meninges are intact forbids an unqualified conclusion.

The meningitis in postvaccinal encephalitis and allied diseases is minimal. However, when it is present, it may be limited to the pia.

4. Seifried, O., and Spatz, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **124**: 317, 1930.

The section shown in figure 3*B* was taken in a case of postvaccinal encephalitis. The pia alone was involved.

Dementia paralytica is also an encephalitis. In this disease, again, the infiltration may be clearly restricted to the pia (fig. 3*C*). Alzheimer [5] and Jakob,[6] who obviously distinguished the parts of the meninges, commented on the preponderance of infiltrating cells in the pia. Freeman [7] regarded the involvement of the pia as secondary to the perivascular infiltration within the depths of the cortex. However, involvement of the pia alone is not universal in this disease. The subarachnoid space and the arachnoid may show changes. This disease illustrates the relative nature of the observation under discussion. After a certain degree of involvement of the pia the infiltration passes beyond this membrane and becomes manifest in the other components of the meninges. In

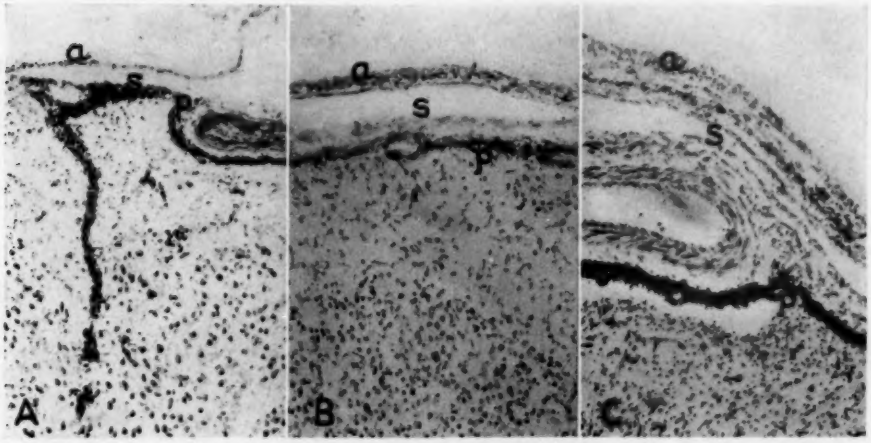

Fig. 3.—*A* is a section of the cortex in a case of Borna disease of the horse. The relationship between the pia and the perivascular infiltration is shown. In the sections illustrated in this figure and in figures 4 and 5, *a* indicates the arachnoid, *s* the subarachnoid space and *p* the pia. *B* is a section of the cortex in a case of postvaccinal encephalitis, and *C*, in a case of dementia paralytica. The cellular infiltration is limited to the tissue adjoining the cortex.

cases of dementia paralytica the long clinical course of the disease may modify the character of the meningitis. Seemingly, however, the cellular infiltration of the pia is primary.

In the noninfectious diseases, such as subacute combined degeneration with which Spielmeyer [1] drew an analogy, there is likewise restriction of the cellular infiltration to the pia.

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5. Alzheimer, A.: Histol. u. histopath. Arb. ü. d. Grosshirnrinde **1**:18, 1904.

6. Jakob, A., in Aschaffenburg, G.: Handbuch der Psychiatrie, Franz Deuticke, Vienna, 1927, vol. 1.

7. Freeman, Walter: Neuropathology, Philadelphia, W. B. Saunders Company. 1933.

In view of the rôle of the pia in encephalomeningitis, it becomes pertinent to know the fate of this membrane in meningo-encephalitis, which may develop in cases of acute purulent meningitis and epidemic cerebrospinal meningitis. In these processes the pia suffers one of three fates:

1. It may be exempt from cellular infiltration, as illustrated in figure 4. Such a condition is more frequent in the spinal cord, partly because the pia is of greater thickness about this organ than about the cerebrum or cerebellum (Weed [8]).

2. The pia may show a reaction pattern differing from that of the subarachnoid space. In figure 5 round cells are shown to predominate in the pia to the exclusion of leukocytes, whereas the subarachnoid space

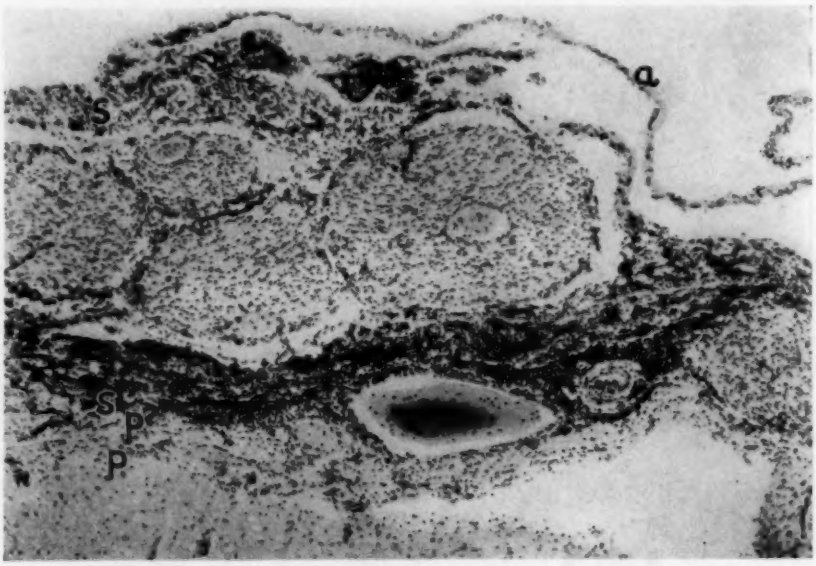

Fig. 4.—Section of the spinal cord in a case of acute purulent meningitis. The pia is exempt from cellular infiltration.

is densely infiltrated with cells of the latter type. Stern,[9] Hassin [10] and Stone [11] have commented on the difference between the reaction pattern of the pia and that of the subarachnoid space in acute epidemic meningitis. Here, again, it is seemingly a question of symptomatic inflammation. The pia reacts in a fashion similar to that of the adventitia of the blood vessels about a focus in poliomyelitis.

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8. Weed, Lewis H., in Penfield, Wilder: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932.

9. Stern, Felix: Arch. f. Psychiat. **58**:216, 1917.

10. Hassin, George B.: Arch. Neurol. & Psychiat. **14**:468, 1925.

11. Stone, Theodore T.: Arch. Neurol. & Psychiat. **23**:106, 1930.

The reaction about the vessel shown in figure 5 corresponds with that in the pia and makes the parallel to the symptomatic inflammation in poliomyelitis more striking. Infiltration of plasma cells and lymphocytes about the vessels as they lie in the superficial layers of the cortex is commonly observed in cases of acute meningitis. It depends to a large extent, seemingly, on the degree of involvement of the pia, that is, on the character of its cellular response.

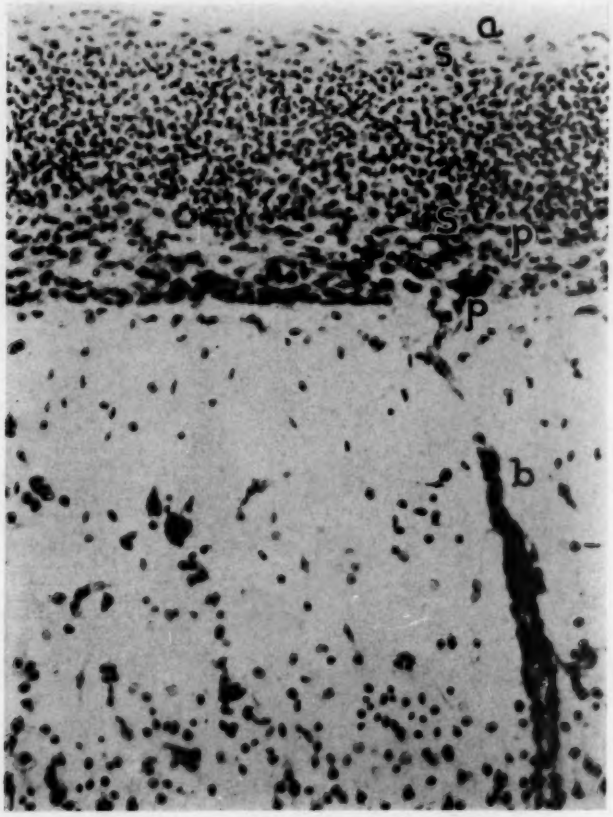

Fig. 5.—Section of the cortex in a case of acute purulent meningitis, showing the difference in the reaction pattern in the components of the meninges. Plasma cells predominate in the pia and about the blood vessel (*b*).

3. The third possible fate of the pia is identical with that of the subarachnoid space. The two meningeal components may show the same reaction. In such an event, the one most commonly observed at autopsy, the infection has penetrated the barrier of the pia. The reaction is also evident in the adventitia of the blood vessels in their superficial course. Leukocytes overshadow the lymphocytes and plasma cells, or they replace

them. The symptomatic inflammation moves farther along the course of the vessels and reaches the superficial layers of the nerve tissue, making complicating encephalitis a reality. Acute meningitis (meningo-encephalitis) is, as Quincke [12] contended, primarily an infection of the subarachnoid space. The other meningeal components are infected only later.

In acute meningitis associative phenomena exist that are not present in encephalomeningitis. The nerve roots lying in the subarachnoid space are surrounded in acute meningitis by infiltrating cells (fig. 4). In encephalomeningitis this condition is not present (fig. 2). So-called ependymitis, or infiltration of the tissues underlying the ependyma of the ventricles, occurs frequently in cases of primary infection of the subarachnoid space, but it is a remote possibility in instances in which the pia is primarily involved. Less pertinent is the existence of foci of ischemic necrosis of different degrees in many cases of acute meningitis, whereas in the reverse condition, encephalomeningitis, such phenomena are not observed.

COMMENT

To summarize briefly, in encephalomeningitis the cellular infiltration of the meninges is predominantly in the pia. In certain forms of meningo-encephalitis the cellular infiltration is predominantly in the subarachnoid space, the pia suffering only secondarily. In explanation of this phenomenon it is desirable, as Spielmeyer elected to do, to seek transitions from the known to the unknown. The surfaces of fasciae of connective tissue or of membranes, of which the pia is an example, are stout barriers against the spread of inflammation. Once this barrier is passed, however, penetration through the fascia to the underlying tissues is not achieved. Rather, the inflammation, if still progressive, spreads in both directions between the parallel fibers of the body of the fascia. That is, the injurious agents are confined and fought in the relatively nonvital tissue composing the fascia—the connective tissue. Penetration through the fascia occurs only when it is overwhelmed.

On the basis of this observation, the next step in the explanation rests on the continuity of many fibers of the pia with those of the adventitia of the blood vessels as they enter the substance of the brain. As Schaltenbrand and Bailey [13] have indicated, this continuity is easily demonstrable with the Perdrau method. In a case of encephalomeningitis when once an agent capable of eliciting a cellular response surmounts the surface barrier of the adventitia of the blood vessel in the neighborhood of a focus, it spreads within the body of the fascia along its longitudinal axis in both directions. If the agent has not become inert

12. Quincke, H.: Deutsche Ztschr. f. Nervenh. **40**:78, 1910.

13. Schaltenbrand, G., and Bailey, P.: J. f. Psychol. u. Neurol. **35**:199, 1928.

before reaching the meninges, it spreads into the meshes of the pia and not into the subarachnoid space because of the continuity of the fibers of the adventitia with those of the pia. Penetration into the subarachnoid space occurs only when the pia is overwhelmed. Bruce and Dawson [14] defined a similar avenue for the movement of lipoids in subacute combined degeneration. They reported movement of the lipoids toward the surface of the spinal cord by way of the perivascular spaces to the inner layer of the pia, from which they possibly move toward the subarachnoid space.

This same explanation clarifies the disparity between the extreme difficulty encountered by an agent in invading the brain in meningoencephalitis and the relative ease with which it spreads into the pia in encephalomeningitis. In acute meningitis cellular infiltration about the blood vessels within the brain and in brain tissue itself is not universal, as might be suspected on contemplating the massive infiltration of the subarachnoid space, but is exceptional. If extension beyond the meninges in the direction of the brain tissue is achieved, the avenue of approach is that of the adventitia of the blood vessels. In acute meningitis, then, the pia is again acting as a protective fascia. The agent cannot spread unhampered along the vessels entering the brain because of the barrier erected by the continuity of the fibers of the pia and adventitia. Once penetration into the body of the pia is achieved, the agent spreads along the meshes of the pia. The avenue to the adventitia of the blood vessels within the brain is then open, again because the fibers of the pia and those of the adventitia are continuous.

In syphilitic and tuberculous meningitis, there is no well demarcated electivity of infiltrating cells for certain meningeal components, especially in the presence of granulomatous tissue. Granulomatous tissue in the meninges, as elsewhere in the body, utterly disregards tissues that usually act as barriers. As Alzheimer [5] pointed out for syphilitic meningitis and Ranke [15] for tuberculous meningitis, invasion into the brain occurs by direct extension and not through the medium of the blood vessels.

Typhus does not appear to fall into the category of encephalomeningitis. Though pictures resembling those of poliomyelitis may be observed in this disease, the infiltration of the subarachnoid space is of equal prominence. Perhaps the free mobility of the mononuclear cells that characterizes this form of meningitis produces this blend of meningeal involvement.

In the preparalytic stages of poliomyelitis, fleeting phagocytosis consisting of polymorphonuclear leukocytes may be demonstrated by lumbar puncture in man and in experimental animals. But the source and func-

14. Bruce, A., and Dawson, J. W.: J. Path. & Bact. **15**:169, 1910.
15. Ranke, O.: Histol. u. histopath. Arb. ü. d. Grosshirnrinde **2**:252, 1908.

tion of the pleocytosis are mysterious. Pette,[16] in material obtained from animals in the preparalytic stage of experimental poliomyelitis, was unable to observe meningitis morphologically. Hurst [17] had previously experienced a similar difficulty. In human material it is the rule to find as an aftermath of leukocytic infiltration a progressive reaction on the part of the fixed tissue. Such a condition does not exist in poliomyelitis. Nor is the pleocytosis a response to the virus in the cerebrospinal fluid, for Hurst and Fairbrother [18] were unable to detect it except under unusual experimental conditions. Whatever may be the explanation of this fleeting leukocytic pleocytosis, it seemingly bears no direct relationship to the pathologic condition of the nerve tissue and hence to the method by which the pia becomes involved. Significant, perhaps, is Pette's [16] reported observation on a monkey of a parallel course between the leukocytosis of the blood and that of the spinal fluid.

The difference between the rôle of the pia in encephalomeningitis and that in meningo-encephalitis condemns the careless use of terminology that has crept into use with respect to the meninges. The expressions "pia" and "pia-arachnoid" have been used synonymously with meninges. It would seem desirable to limit those terms to their original definitions.

SUMMARY

The cellular infiltration of the meninges present in certain encephalomeningitic processes is essentially limited to one component, the pia.

This observation augments Spielmeyer's histologic definition of encephalomeningitis.

This infiltration, having the character of the "symptomatic inflammation" of Spielmeyer, is secondary to the encephalitic process.

In purulent meningitis the seat of the inflammation is primarily the subarachnoid space, the pia evidencing merely symptomatic inflammation.

The pia is an effective barrier to the invasion of infection into the brain substance itself.

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16. Pette, H.; Demme, H., and Környey, S.: Deutsche Ztschr. f. Nervenh. **128**: 125, 1932.

17. Hurst, E. Weston: J. Path. & Bact. **32**:457, 1929.

18. Hurst, E. Weston, and Fairbrother, R. W.: J. Path. & Bact. **33**:17, 1930.

OSTEOBLASTOMA OF THE KIDNEY, HISTOLOGICALLY IDENTICAL WITH OSTEOGENIC SARCOMA

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AND
FENN E. POOLE, M.D.
LOS ANGELES

Primary sarcoma of the kidney is a rare lesion. True bone formation in the kidney is a phenomenon so unexpected as to compel an inquiry into the method of its accomplishment. We are reporting a tumor of the left kidney, apparently a primary renal sarcoma, which metastasized to the opposite kidney, the liver and the small bowel. The tumors produced in both kidneys underwent extensive ossification.

Many theories have been advanced to explain the occurrence of bone in soft tissue. They may be classified succinctly as (1) the periosteal theory, (2) the neoplastic theory and (3) the metaplastic theory.

The periosteal theory has lost caste. It may account for osseous spurs directly connected with bone, but it is difficult to believe that periosteum plays any part in bone formation in old tuberculous lymph nodes, in uterine fibromyomas, in fibrosed areas in the ovary, in arteries and cardiac valves, in old cerebral hemorrhages, in hypernephromas and in laparotomy scars.

The neoplastic theory is also inapplicable in the great majority of instances. T. B. Mallory [1] classified the condition in two of the cases in his series, as well as that in case 2 of Rhoads and Blumgart,[2] as "primarily neoplastic," though he recognized that the condition in these cases was consonant with the theory of Leriche and Policard,[3] who expressed the belief that bone in a tumor represents metaplasia within a neoplasm.

Perhaps all bone, normal or abnormal, is in the broader sense of the word metaplastic in origin. This view is not new. Virchow [4] expressed the belief that all bone and cartilage arose from fibroblasts by a process of metaplasia. In 1914, F. B. Mallory [5] wrote:

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1. Mallory, T. B.: Am. J. Path. (supp.) **9**:765, 1933.
2. Rhoads, C. P., and Blumgart, H.: Am. J. Path. **4**:363, 1928.
3. Leriche, R., and Policard, A.: The Normal and Pathological Physiology of Bone, translated by Moore and Key, St. Louis, C. V. Mosby Company, 1928.
4. Virchow, R.: Virchows Arch. f. path. Anat. **97**:410, 1884.
5. Mallory, F. B.: The Principles of Pathologic Histology. Philadelphia, W. B. Saunders Company, 1914, p. 276.

The ordinary connective tissue cell, the fibroblast, produces fibroglia and collagen fibrils. It has inherent in it, however, the property of being able to manufacture certain other substances. . . . If the fibroblast produces in addition to the fibrils a dense homogeneous intercellular substance known as osseomucin in which lime salts tend to be deposited, it is called a bone cell. Bone cells may arise directly from fibroblasts or from an intermediate cell known as an osteoblast.

Recently, Leriche and Policard have supplied convincing experimental evidence in support of the metaplastic theory of osteogenesis. To them fibrous tissue, cartilage and bone are all end-products of a common mesoblastic stem. This means not that they may directly interchange identities but that under certain environmental conditions any connective tissue may revert or dedifferentiate into an embryonic state, and when this occurs fibroblasts may become osteoblasts and form true bone. Greig,[6] "though brought up in the faith that periosteum was osteogenetic," abandoned the periosteal view completely in favor of the metaplastic.

The metaplastic theory of osteogenesis does violence to none of the ascertained facts and offers a rational explanation of the normal and abnormal production of bone. It seems the only available explanation of the type of extraskeletal ossification in the case which we are reporting.

REPORT OF A CASE

A white man, aged 76, entered the Los Angeles County Hospital on June 13, 1932, complaining of frequency of urination, nocturia and dysuria of one year's duration. He had noted blood in his urine four times during the previous year; the hematuria was not accompanied by pain. No symptoms referable to systems other than the genito-urinary tract could be found. The family history was irrelevant.

The patient was well developed and well nourished. The temperature was 98.6 F.; the pulse rate was 80, and the respiratory rate was 18. The blood pressure was 140 systolic and 60 diastolic.

The head, neck, chest, abdomen and extremities were normal on physical examination. The rectal sphincter tone was good. The prostate was somewhat enlarged, resilient, smooth and not fixed. Both testicles were normal to palpation. No lymph nodes were palpably enlarged, and there was no evidence of abnormalities of the bones.

The Wassermann and Kahn reactions of the blood were negative. The total nonprotein nitrogen content was 26 mg. per hundred cubic centimeters of blood; the amount of preformed creatinine was 1.1 mg. per hundred cubic centimeters of blood. Urinalysis revealed albumin (+) and pus cells (+++), and a stained smear of urinary sediment showed many gram-negative rods. The phenolsulphonphthalein test of renal function, repeated at varying intervals over a six week period, gave the following results: The average time of appearance was five minutes; the average excretion of dye was 55 per cent in the first thirty minutes and 15 per cent in the second thirty minutes.

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6. Greig, D. N.: Clinical Observations of the Surgical Pathology of Bone, Edinburgh, Oliver & Boyd, 1931.

Flat roentgenograms of the kidneys, ureters and bladder were reported, on June 16, 1932, as showing the kidneys normal in size, shape and position, with no calculi apparent in any part of the urogenital tract. These roentgenograms were rechecked, and the report was confirmed after autopsy.

The diagnosis of benign adenoma of the prostate was confirmed cystoscopically.

The first stage of a prostatectomy was performed on June 28 and the second stage on August 2. The microscopic report on the tissue removed was "fibro-adenomatous hyperplasia of the prostate."

The patient made an uneventful recovery and left the hospital on September 23. He remained active until March 1934, when he began to complain of occasional vague abdominal pain and distress after meals. On April 11 he cried out with sudden severe abdominal pain, which was followed by vomiting. The vomiting became progressively worse.

Fig. 1.—Hemisections of the kidneys, showing the left kidney completely replaced by tumor tissue and the right kidney containing one circumscribed metastatic nodule.

The patient was hospitalized again on April 15. He was moribund, with definite signs of generalized peritonitis, the etiology of which was indeterminable. Death occurred on April 18.

Autopsy was performed forty-eight hours after death. The body had been embalmed and appeared to be that of a well developed and fairly well nourished white man. There was no evidence of jaundice; no lymph nodes were palpable, and there was no external evidence of any lesion of the skeletal bones.

The pleural membranes were smooth and glistening, and there was no fluid in the pleural cavities. The cut surfaces of the lungs revealed passive congestion in the posterior aspects, and there were bronchopneumonic patches in the lower lobes.

The pericardium was smooth and glistening. The heart weighed about 400 Gm., and the myocardium showed no indication of infarction or of gross scarring. The valves were normal. The coronary vessels showed little sclerosis.

The abdominal cavity was filled with 3.5 liters of foul-smelling, feculent fluid, and all the loops of the bowel were plastered together by a fibrinopurulent exudate. A large mass in the pelvis was seen to be a hard, spheroid, yellowish-white neoplasm, 8 cm. in diameter, situated in the mesentery near its attachment to the small bowel 2 feet (60.96 cm.) from the duodenojejunal junction. The weight of the tumor had apparently carried the loop of intestine down into the pelvis. Several similar but smaller nodules were scattered throughout the remainder of the mesentery, and four flat tumors, ranging from 3 to 5 cm. in diameter and

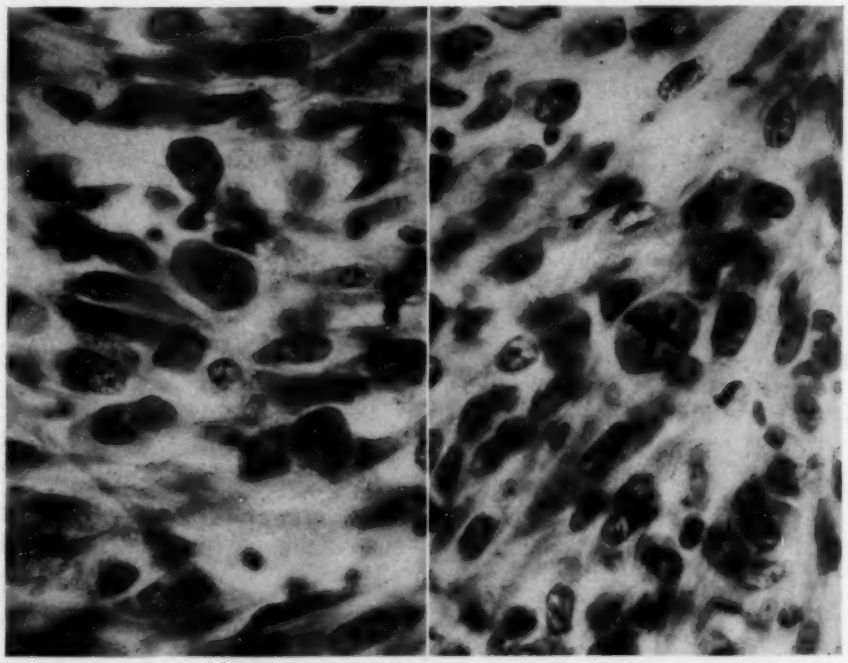

Fig. 2.—Sections (× 180) from metastatic lesions in the liver, showing the fibroblastic nature of this tumor and examples of mitoses.

from 1 to 2 cm. in thickness, were present in the wall of the small bowel. These tumors were firm, grayish-white, smooth elevations on the serosal surface. Each lesion seemed to project into the lumen of the bowel, mostly from its periphery, thus forming a peculiar fluted tumor with a depressed center. The largest, situated about 3 feet (91.44 cm.) from the ileocecal valve, was the site of a perforation 0.5 cm. in diameter. This was obviously the source of the peritonitis.

The liver was enlarged and contained several hard, white, spheroid tumors ranging from 2 to 5 cm. in diameter. Each tumor seemed well circumscribed and shelled out readily as though encapsulated, and on the cut surface the center was seen to be somewhat honeycombed and contained some light yellowish mucoid material.

The spleen and pancreas appeared normal.

The left kidney was not enlarged and was almost stony hard to palpation. Its capsule could not be stripped. The external surface was somewhat nodular, but the general contour of the kidney was not altered. It cut with great resistance, and occasionally the knife seemed to strike calcareous material. On section the parenchymal architecture was completely obliterated, the cortex and medulla being replaced by hard, gray-white, fairly homogeneous tissue. The right kidney was of normal size and shape, and its capsule stripped readily, leaving a smooth surface. The parenchyma appeared normal except for a well circumscribed spheroid nodule about 3 cm. in diameter, which in hardness and in the appearance of its cut surface resembled the neoplasm of the left kidney (fig. 1). The ureters and bladder presented no change. The prostate had been removed.

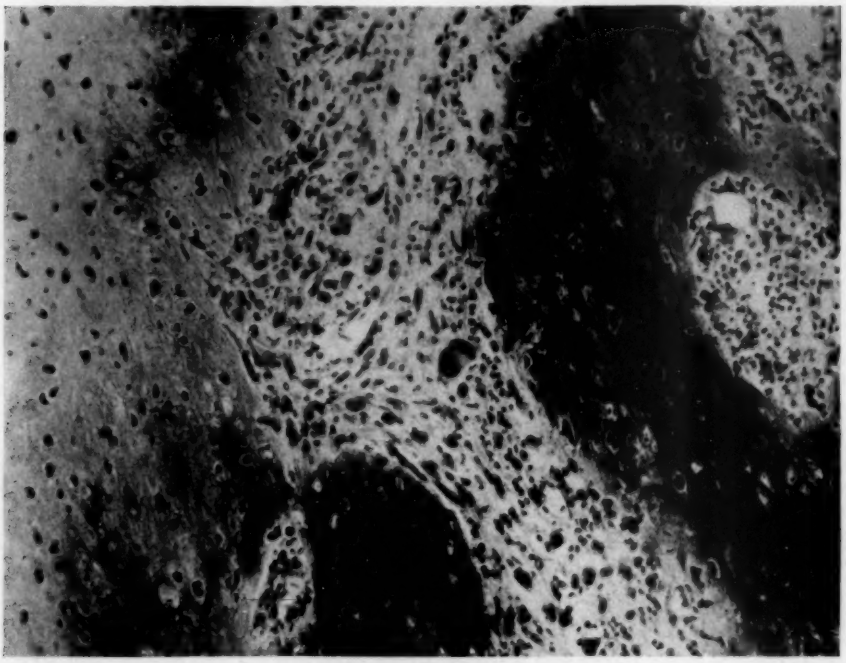

Fig. 3.—Section (× 130) from the original renal tumor, showing calcification of osteoid tissue. A tumor giant cell is shown.

Microscopic sections from the left kidney showed almost complete replacement of the renal structure by a neoplasm composed of oval and spindle-shaped mesodermal cells, which appeared identical with those seen in cases of osteogenic sarcoma. There was a moderate amount of collagenic stroma, and many multinucleated giant cells of the tumor type were scattered throughout the growth. The nuclei were hyperchromatic, and pale-staining cytoplasm was fairly abundant. Mitotic figures were numerous and included examples of mitoses in three and four separate planes (fig. 2). Scattered irregularly throughout the sections was considerable osteoid tissue, some of which showed deposition of calcium and was undergoing calcification (fig. 3). There was a direct transformation from the cellular tumor tissue to osteoid tissue, with no intervening cartilaginous tissue (fig. 4). Along some borders of the osteoid tissue were seen rows of osteoblasts which were smaller and rounder than most of the tumor cells.

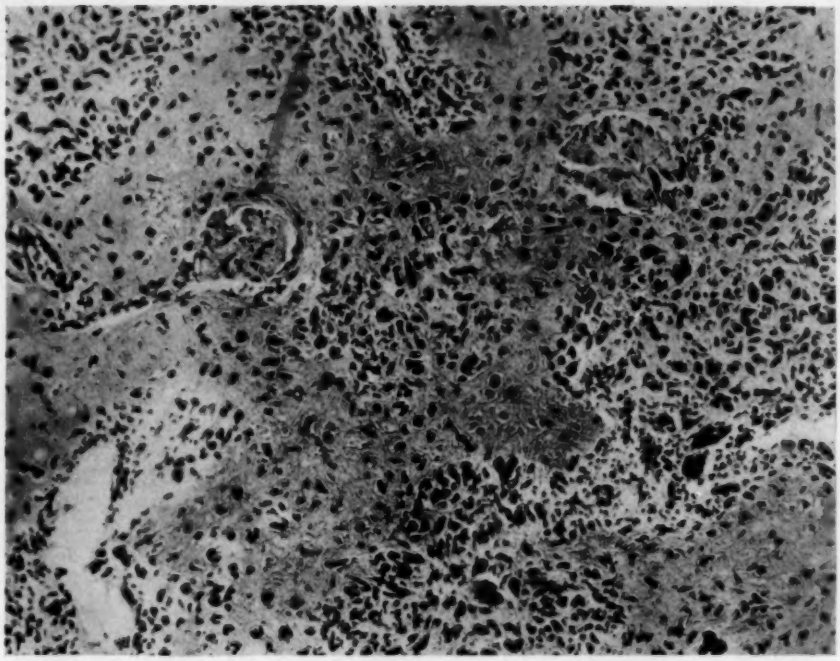

Fig. 4.—Section (× 130) from the original renal tumor, showing the relationship of the tumor and osteoid tissue. Note the glomeruli surrounded by osteoid tissue.

Fig. 5.—Section (× 130) from the metastatic growth in the right kidney, showing its circumscribed character.

The small amount of recognizable renal parenchyma which remained showed fibrosis and hyalinization and, in a few areas, necrosis involving tumor tissue as well as the renal tissue. There were scattered collections of lymphocytes and plasma cells, as well as collections of golden-brown pigment, probably indicating a low grade inflammatory reaction and old hemorrhage. Several large thrombosed blood vessels were present, some of the thrombi being composed of, or infiltrated with, tumor tissue. The capsule of the kidney and the perirenal fat had been invaded in some places by the neoplasm, but no osteoid tissue was seen in these areas.

A section taken from the right kidney showed a neoplastic process which was identical with the opposite renal growth, except that it was sharply circumscribed from the surrounding kidney structure (fig. 5).

The metastatic lesions in the liver and bowel were also well circumscribed and were composed uniformly of spindle-shaped mesoblastic cells with many mitotic figures, but without any attempt at bone formation (fig. 2).

There were no lesions in the other viscera except a rather confluent type of bronchopneumonia.

COMMENT

Confronted at autopsy with tumor formation in both kidneys and in the liver and small bowel, we naturally wondered where the primary lesion was. Obviously the tumors in the right kidney and in the liver and bowel were metastatic. The left kidney was practically completely replaced by tumor tissue, whereas the other lesions were smaller and more circumscribed. In the absence of any other discoverable tumor, the left kidney seemed the only possible site of the primary lesion.

The extraordinary hardness of the left kidney provoked a surmise that it might contain bone, and this led us to wonder whether such a condition could be secondary to an osteogenic sarcoma of skeletal bone. We were not allowed to remove the bones for careful examination, but no such tumor was visible to the eye or palpable to the finger. The patient had been seen occasionally in the outpatient clinic (to and from which he had traveled unaided) and had been carefully examined in the hospital four days before death, and at no time had he complained of symptoms or exhibited signs which might point to an osseous lesion.

At autopsy the possibility of a primary skeletal sarcoma seemed to us incredible for two reasons: first, the age of the patient and, second, the absence of any indication of a skeletal osseous tumor, which, if present, would ordinarily have reached conspicuous dimensions by the time that metastases and death had occurred. Geschickter and Copeland [7] cited two examples of osteogenic sarcoma in persons over 70 years of age, but this must be extremely rare. Ewing [8] stated that "the age limits are between 10 and 50 years."

7. Geschickter, C. F., and Copeland, M. M.: Am. J. Cancer 210, 1931.

8. Ewing, James: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 300.

The gross appearance of the two kidneys (fig. 1) strongly supports our acceptation of the lesion of the left kidney as a primary osteogenic sarcoma. Speaking of renal sarcoma, Kaufmann [9] stated that "secondary sarcomas usually form rounded, sharply demarcated nodules." This observation, which every pathologist of experience will recognize as a fair characterization of the average metastatic sarcoma, exactly describes the nodule in the right kidney. The left kidney, however, did not contain a circumscribed neoplasm but was completely replaced with tumor tissue, and it is difficult to believe that this could represent a secondary growth. This view is slightly strengthened by the common clinicopathologic observation that primary tumor of the kidney (and also unattached retroperitoneal tumor the histogenesis of which is probably from remnants of the urogenital apparatus [10]) occurs more frequently on the left side. However, such an observation is indirect evidence at best, and at worst it might not prove to be factual in a series of cases large enough to justify deductions.

It is impossible, therefore, to prove indisputably that this patient did not have a primary osseous sarcoma from which all the tumors seen by us were metastatic. However, even if one assumes the presence of such a lesion one comes no nearer to an intelligent interpretation of the renal phenomena. Many physicians are deceived, partly by the very term "osteogenic sarcoma," into supposing that a malignant tumor of bone frequently produces bone in its metastases. Their a priori reasoning is that when a carcinoma metastasizes another carcinoma is formed and when a sarcoma metastasizes another sarcoma is formed and that therefore when an osteosarcoma metastasizes one should expect to find other osteosarcomas. The analogy is fallacious, for while there is apparently a carcinoma cell or a sarcoma cell which when transferred may reproduce, respectively, a carcinoma or a sarcoma, there is no such thing as a bone cell which may be depended on to reproduce bone. Greig [6] has not been able to find a single instance of bone formation in a metatasis from osteogenic sarcoma in the museum of the Royal College of Surgeons of Edinburgh. He contended that if osteosarcoma cells possessed in themselves the power to form bone, all the metastases, wherever produced, should be osseous. His view was stated as follows:

> A metastasis from a sarcoma reproduces its original cells more or less accurately, but it never produces bone unless its vascularity taps some supply of calcium whether it be a physiological reservoir, such as bone, or a mere heterotopic collection of calcium previously accumulated. If the cells of a sarcoma were bone forming, the metastases would certainly be, at least frequently, osseous, and if osseous in one organ ought to be osseous in all. This they certainly are

9. Kaufmann, E.: Pathology for Students and Practitioners, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 2, p. 1394.

10. Hansmann, G. H., and Budd, J. W.: Am. J. Path. **7**:631, 1931.

not. It is significant that no professional pathologist, even of wide experience, with whom I have discussed the matter, can recall from his own experience a single case in which even one metastasis from a periosteal or endosteal "osteogenic" sarcoma contained bone. The fallacy of the frequency of its occurrence seems to be promoted from text-book to text-book on the strength of some well-nigh unique specimen preserved on account of its rarity. When on rare occasions a metastasis shows, apart from bone, a bony structure, it is significant that that metastasis is situated where calcification is known to occur.

In harmony with the theories of Greig and Leriche and Policard, it seems generally true that heterotopic ossification occurs mainly in situations where calcareous collections are frequently found. Thus, it has been reported in the lungs (by far the most common location), liver, spleen, lymph nodes, thyroid, brain and meninges. Hektoen [11] has "seen bone in secondary osteogenic sarcoma of the heart." In an unusual case, reported from this laboratory by Harding and Courville,[12] there were bone formation in the liver and lung and metatases to the brain from an osteogenic sarcoma of the femur. A nodule in the left central region revealed only the earliest evidences of calcium deposition, while another nodule, which was in contact with dura and bone, contained such an abundance of calcium as to make it roentgenographically visible. This argues in favor of the probability that a local, preferably contiguous, source of calcium greatly facilitates the production of adult bone.

RENAL OSTEOGENESIS

In our case ossification occurred only in the renal neoplasms. So far as we know, an instance of primary renal osteogenic sarcoma has never before been recorded, and bone-forming metastases in the kidney must be extremely rare. This is somewhat surprising in view of the apparent ready availability of calcium in the urogenital system and especially in view of the numerous experimental demonstrations of a peculiar osteogenic proclivity in some of the urogenital tissues. In 1923, Phemister [13] wrote:

There exists a tendency to ossification in the organs of the urinary tract, under certain experimental and pathological conditions, which is greater than that in any other system of organs. Some of this ossification appears to be related to the direct and continuous action of the urine upon altered tissue bordering on the urinary channel. That in the kidney results from the fact that it is an end organ, and interference with its blood supply is followed by degeneration, which creates a field for calcification and ossification that is more favorable than that produced in other organs. Experimentally, bone has been produced in ureter, bladder, and kidney.

11. Hektoen, Ludvig: Personal communication to the authors.
12. Harding, W. G., and Courville, C. B.: Am. J. Cancer **21**:787, 1934.
13. Phemister, D. B.: Ann. Surg. **78**:239, 1923.

In this article Phemister described ossification in three kidney stones attached to the renal pelvis (Hellström [14] has since reported similar cases) and pointed out the resemblance between bone, calcified areas and urinary stone. He proposed the possibility of a common factor, perhaps the urinary acidity, which might influence all three processes (calcification, ossification and stone formation) under different environmental conditions. In the succeeding years a great deal of valuable work was performed, notably by Huggins,[15] who produced bone by transplanting epithelium of the renal pelvis to certain parietal fasciae and to muscle and synovial membrane in dogs, thus demonstrating that the mucosa of the renal pelvis possesses a peculiar capacity for stimulating bone formation.

A number of instances of renal ossification have been recorded in which (as in our case) neither stone nor pelvic epithelium appears to have played any part. Thus, Phemister [13] has seen partial replacement by bone of calcified areas in cases of tuberculosis of the kidney and of hypernephroma. Recently Moro [16] reported "a rare case of ossifying Grawitz tumor." As might be expected, bone formation is seen more commonly in mixed renal tumors than in any other renal lesion. Many clinicians do not appreciate the fact that these tumors are by no means strictly limited to infants and children. Ssoroko [17] has described mixed tumors in two adults aged 58 and 38, in one of which osseous tissue was noted. A few years ago Clay [18] reported a mixed tumor of the kidney in a patient aged 80. In view of these occurrences, most pathologists, on being told of a bone-containing renal tumor, will wish to assure themselves, no matter how elderly the patient, that the lesion is not a mixed tumor. Grossly and microscopically, the neoplasm which we have described bore no resemblance to the typical mixed tumor, which is usually a growth of unusually large dimensions, its weight often being from 5 to 20 per cent of the total body weight. Microscopically, a mixed tumor shows an extremely varied composition. Smooth and striated muscle with connective tissue of different types, cartilage and even bone, and round cell sarcomatous tissue, often with alveolar structure, may be seen bound together in a framework of embryonic connective tissue.

NOMENCLATURE

A nonskeletal osteogenic sarcoma which seems to parallel the tumor in our case in every respect save location has recently been described

14. Hellström, J.: Ztschr. f. Urol. **25**:401, 1931.
15. Huggins, C. B.: Arch. Surg. **22**:377, 1931; **27**:203, 1933.
16. Moro, N.: Deutsche Ztschr. f. Chir. **240**:642, 1933.
17. Ssoroko, N. K.: Ztschr. f. Urol. **25**:738, 1931.
18. Clay, John: Brit. M. J. **2**:1083, 1930.

by Broders and Pemberton.[19] The tumor was discovered in a thyroid gland and represents the first of its kind noted in 40,000 thyroid glands examined in the pathologic laboratory at the Mayo Clinic. Broders and Pemberton used the term "primary osteogenic sarcoma of the thyroid."

Terminologically, Mallory [1] was more conservative and used the phrase "bone- and cartilage-containing tumors." Rhoads and Blumgart [2] employed a very lucid title, "two osteoblastomas not connected with bone, histologically identical with osteogenic sarcoma, and clinically benign" (in the sense that removal of the tumor was followed by apparent cure).

SUMMARY

A man, 78 years old, was observed at autopsy to have neoplasms involving both kidneys, the liver and the small bowel, where one of the tumors perforated, causing peritonitis and death.

Both renal tumors contained bone. The left kidney was completely replaced by tumor tissue, largely osseous, with irregular deposition of calcium. The right kidney contained only a small well circumscribed nodule.

In the liver and bowel the tumors microscopically were fibrosarcoma. In the kidneys they appeared to be primarily fibrosarcoma which, under the influence of an unknown factor (perhaps phosphatase), underwent dedifferentiation into embryonic fibroblasts which produced bone. This seems to represent an essentially metaplastic process within a neoplasm.

The lesion of the left kidney appeared to be the parent tumor. Histologically, it resembled osteogenic sarcoma, and it might not be inexact to call it a primary osteogenic sarcoma of the kidney.

19. Broders, A. C., and Pemberton, J. de J.: Surg., Gynec. & Obst. **58**:100, 1934.

TUMORS OF THE OVARY

WITH SPECIAL REFERENCE TO THE BENIGN FIBRO-EPITHELIOMA (BRENNER TUMOR)

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CHICAGO

Until the present time the histogenesis of primary tumors of the ovary has been the subject of much controversy, which has received additional stimulation by the discovery that some of these tumors are characterized by a distinct hormonal influence on their bearer. Whereas in former years one was satisfied to distinguish between epithelial and mesenchymal and benign and malignant neoplasms of the ovary, Meyer recognized a classification which was based on biologic and embryologic criteria, thus opening a new field to further investigative studies. According to Meyer, one can distinguish between ovarian tumors which have a masculating or a feminizing effect and tumors which do not display endocrine activity. To the latter type belongs the Brenner tumor, which is one of the more discussed new growths in the literature on ovarian neoplasms.

In reviewing the literature on this type of tumor, Meyer [1] in 1932 found reports of eighteen cases and added four of his own. Plaut [2] the following year reported eight cases which came under his observation, making a total of thirty cases. In the last few years reports of additional cases have been published, especially in the German literature (Abraham,[3] Freund,[4] Schiller [5] and others), while in the American and British literature little attention has been devoted to this interesting type of ovarian new growth. Recently, an ovarian tumor of the Brenner type was submitted for examination to the surgicopathologic laboratory of the Cook County Hospital which was striking because of its unusually large size. While the study of this tumor was in progress, two other similar tumors were encountered incidentally at autopsy.

REPORT OF CASES

CASE 1.—*History.*—A nulliparous Negress, aged 45, entered the Cook County Hospital because of a slowly enlarging swelling of the abdomen which she had

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1. Meyer, R.: Arch. f. Gynäk. **148**:541, 1932.
2. Plaut, O.: Arch. f. Gynäk. **153**:97, 1933.
3. Abraham, Ernest G.: Arch. f. Gynäk. **154**:565, 1933.
4. Freund, R.: Arch. f. Gynäk. **155**:67, 1934.
5. Schiller, W.: Arch. f. Gynäk. **157**:65, 1934.

noticed for the past three years. Diarrhea had been present for the past two or three weeks and cough for four months. During the past year the ankles and legs were swollen. The menses had ceased two years before, and there had been no bleeding from the vagina since that time. On physical examination the outstanding point of interest was the enlargement of the abdomen, which was of

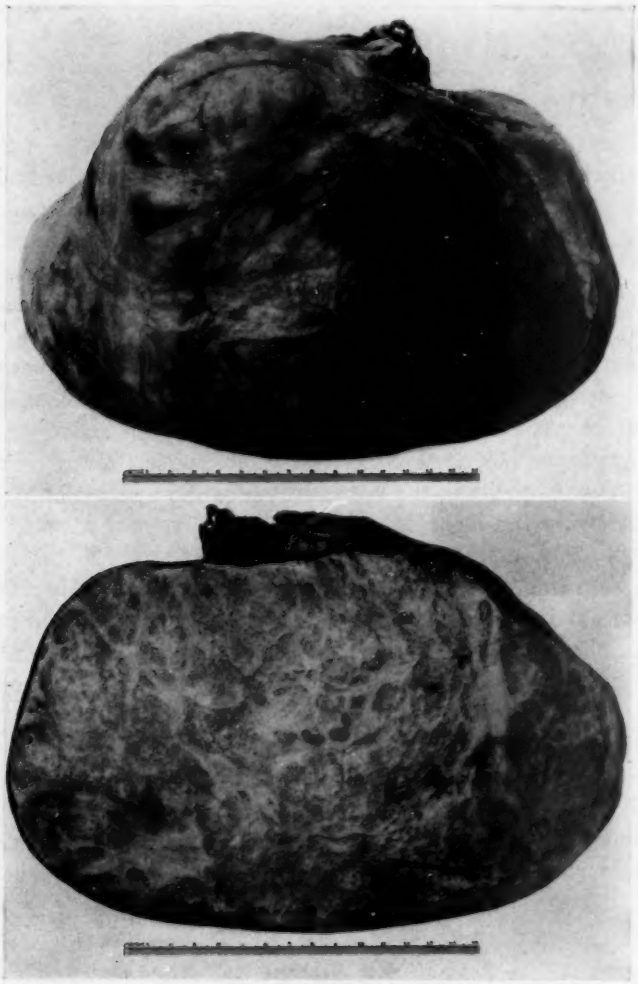

Fig. 1.—Outer and cut surfaces of the tumor of the left ovary removed at operation.

the size observed in full term pregnancy. The enlargement was due to a ballotable, freely movable hard mass. On vaginal examination movement of the tumor was not transmitted to the cervix.

Operation.—A mass weighing 15 pounds (6,803.88 Gm.) was disclosed, located in the region of the left ovary and attached to the fundus of the uterus by fine

adhesions. The tumor, the fundus of the uterus and the left fallopian tube were removed. The patient made an uneventful recovery and was discharged from the hospital ten days after the operation. She did not return.

Gross Description.—The specimen removed at operation included the uterus amputated above the cervix, which measured 6 by 5 by 3 cm. The outer surface was smooth. The wall was 1.5 cm. thick, and the endometrium was gray-tan and smooth. The left fallopian tube was slightly thickened, and the fimbriated end was occluded. In addition, there was an ovoid mass (fig. 1) measuring 25 by

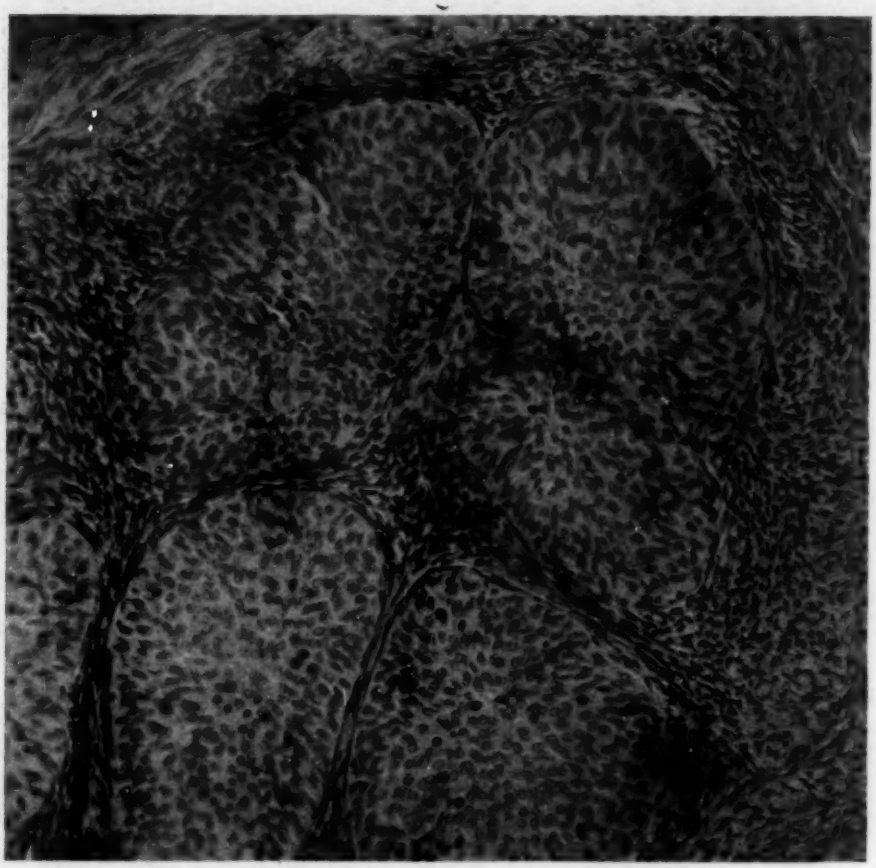

Fig. 2.—Photomicrograph (× 150) of a section of the surgical specimen stained with hematoxylin and eosin. The large polygonal cells grouped in nests are evident.

28 by 10 cm. in diameter. The outer surface of this mass was yellowish gray-white with smooth, lobular elevations of as much as 2 cm. The mass was very firm. The surfaces made by cutting were gray-tan streaked with bands of lighter gray tissue, which divided the field into circumscribed areas measuring as much as 5 cm. in diameter. In some portions cavities were hollowed out to a depth of 2 cm. and were filled with yellow-gray gelatinous material.

Histologic Description (figs. 2 and 3).—Firm Portions of the Tumor: Embedded in an ample stroma of dense fibrillar connective tissue that stained bright purple-red with the Van Gieson method were round and oval nests of cells, which varied from 200 to 400 microns in diameter. These nests were composed of large polygonal cells with a distinct rim of cytoplasm, which was either pale and vacuolated or finely granular and showed a distinct affinity for the acid stains. The nuclei were round and centrally located and possessed a thin membrane. Their chromatin

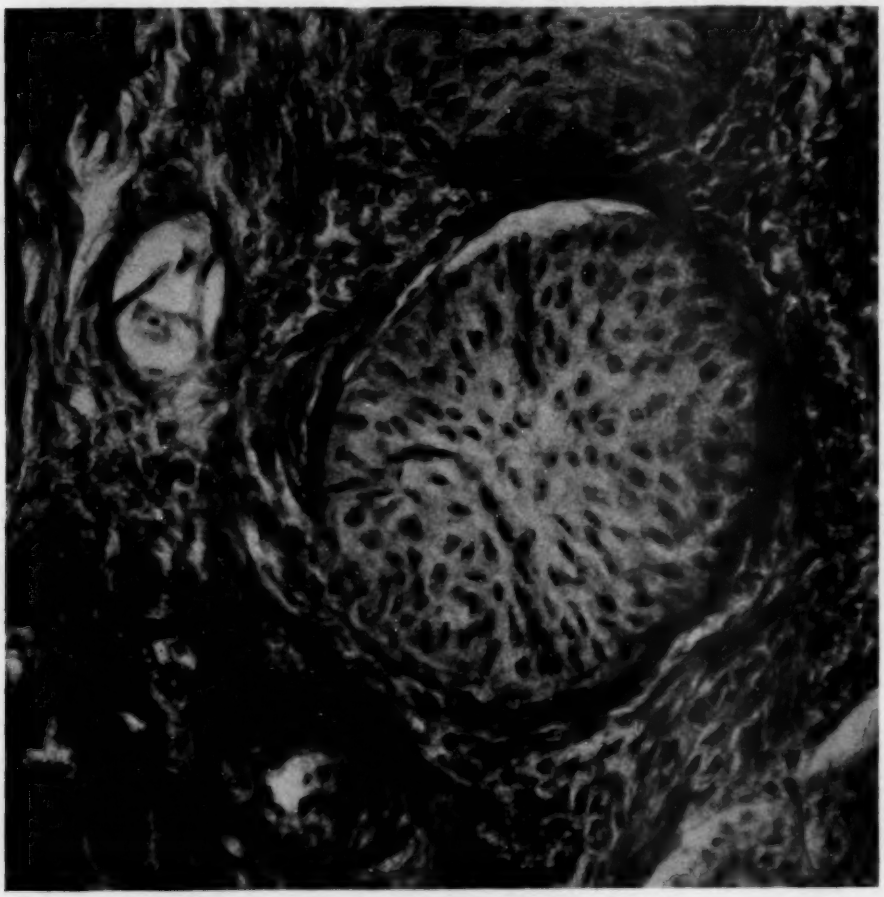

Fig. 3.—Photomicrograph (× 300) of a section of the surgical specimen stained with phosphotungstic-acid hematoxylin. There is a thick mantle of connective tissue about the groups of cells.

was coarsely granular, and there was usually a single, distinct nucleolus. In preparations stained with sudan III a few cells were observed to contain single small fat granules. A stain for glycogen failed to demonstrate its presence, but since the material was fixed in a solution of formaldehyde the negative result was not conclusive. Many nests of cells showed central liquefaction, which gradually led to the transformation of the solid nests into cavities lined by several layers of

the characteristic cells. From the stroma the epithelial formations were separated by a mantle of hyalinized connective tissue that stained pinkish red with the Van Gieson method. The blood vessels were few.

Soft Portions of the Tumor: The structure of the softer portions differed from the picture just described in edematous loosening of the stroma and change in the character of the epithelial structures. The epithelial cells showed a tendency to arrange themselves in a glandlike fashion. Some glands were lined by a single, regular layer of columnar epithelial cells, which had clear cytoplasm and basal nuclei, while other glands showed several layers of low cuboidal epithelium. The lumens formed by these glands sometimes contained granular débris.

Case 2.—In a Negress, aged 49, who died of lobar pneumonia in the stage of red and gray hepatization, a nodule 1.5 by 1.3 by 1 cm. was noted in the right ovary. The nodule was round, firm and light yellow-gray. Examination of the uterus revealed fibromyomas.

Microscopic examination of the nodule showed a dense stroma consisting of interwoven bundles of fibrocytes and collagen fibers. In places there were a few deposits of calcium. Embedded in the stroma were a moderate number of round to oval nests of cells. The cells were large and had abundant, clear cytoplasm and oval, vesicular nuclei with occasional nucleoli. In the periphery, the cells were occasionally arranged in a basal manner. The node was surrounded by a narrow capsule of connective tissue.

Case 3.—A Negress, aged 50, died of a dissecting aneurysm of the aorta which had ruptured into the pericardial sac. The autopsy also revealed an irregular, very firm grayish-white nodule 1 cm. in diameter on the surface of the right ovary. This was composed of dense, cellular, fibrous stroma which separated nests of moderately sized, pale epithelial cells with oval, vesicular nuclei. About each nest of cells, there was a mantle of hyalinized connective tissue. Deposits of calcium were present in moderate amount.

COMMENT

During embryonal development three successive sets of urogenital tubules are formed: the pronephros, which does not persist; the mesonephros, which forms the wolffian body with its ducts and the primitive kidney, and the metanephros, part of which is derived from the mesonephros, which differentiates into the permanent kidney. The mesonephros is intimately connected with the genital apparatus. It forms in its caudal portion the wolffian ducts, which persist in the female only as the rudimentary epoophoron and the rete ovarii. The genital ridge is a mass of mesenchymal cells which arises medial to these sets of tubules and ultimately gives rise to the sex gland, both in the male and in the female. The mass of cells migrates to the pelvis and comes into close contact with the wolffian body. The wolffian body furnishes the connective tissue for the ovary, while the cells of the genital ridge form a surface layer over it. According to Winiwarter,[6] three successive proliferations of the surface germinal epithelium occur. The first proliferation of the germinal epithelium penetrates into the mass of cells

6. Winiwarter, cited by Popoff, N. W.: Arch. Path. **9**:31, 1930.

to form the medullary cords. These cords degenerate in the female, but in the male they differentiate into the seminiferous tubules. The second proliferation results in the Pflüger cords and tubules, which produce primordial follicles without ovules and degenerate after birth; in the third proliferation masses of cells are formed which become the graafian follicles with ova. Lane-Claypon [7] has shown that the interstitial cells of the ovary are formed from the other cells originating from the germinal ridge. Connective tissue cells derived from the wolffian body surround these masses of cells and cut them off from one another and from the surface epithelium.

If one considers the intricate development of the ovary and the fact that practically throughout life the ovary is affected by many variable factors, one will readily appreciate the complexity of the new growths which may originate in this organ. It is not within the scope of this paper to discuss all the types of ovarian tumors, but it may be of interest to review briefly the most significant.

For a long time, certain tumors of the ovary presented themselves with such variable pictures that classification and understanding were difficult. Meyer considerably clarified the problem by classifying these tumors on the basis of the hormonal effect on the patient seen as a change in the secondary sexual characters. On cytologic grounds, also, great similarity appeared between the tumors of like hormonal expression.

In the original classification of Meyer there are two groups. The first group is formed of the granulosa cell tumors, which are most generally believed to arise from embryonic rests of ovarian parenchyma, which are closely related to the granulosa cells. Gottschalk assumed that the tumors arise from embryonic follicle cells, while Ujma [8] expressed the opinion that they come from ripe follicle cells. It is unlikely that these tumors develop from adult follicle cells because the life of follicular tissue is so entirely dependent on the life of the ovum which it surrounds. The embryonic rests are probably cells of the Pflüger cords which have not degenerated as they ordinarily do and which are stimulated to grow by an as yet unknown stimulus. Evidence in favor of this theory is derived from the fact that proliferative changes in the follicles are never observed, even in an ovary with inflammatory and proliferative changes. It is also of significance that early granulosa cell tumors are always located in the hilus of the ovary, at a distance from the cortex (TeLinde [9]). Furthermore, granulosa cell tumors occur in women much more frequently after the menopause than during the active life of the follicular cells. Clinically, the majority of these tumors, in either their folliculoid or their diffuse form, display a feminizing action. Thus,

7. Lane-Claypon, cited by Goodall, J. R.: Surg., Gynec. & Obst. **30**:249, 1920.
8. Ujma, cited by Plate, W. P.: Arch. f. Gynäk. **151**:26, 1932.
9. TeLinde, R. W.: Am. J. Obst. & Gynec. **20**:552, 1930.

they cause hyperplasia of the uterus with menorrhagia. The breasts frequently hypertrophy also. In children they cause precocious puberty, and in young women they are associated with amenorrhea.

A second group of feminizing tumors are those which, according to Löffler and Priesel,[10] arise from the cells of the theca interna. Those authors named tumors of this type fibroma thecocellulare xanthomatodes ovarii. In the following year Melnick and Kanter [11] reported two cases, which with six cases originally reported by Löffler and Priesel and four others added later [12] comprise the entire number of reported cases of tumors of this type. These theca cell tumors, like the granulosa cell tumors to which they are undoubtedly related, produce glandular hyperplasia of the endometrium, postmenopausal bleeding and, in young women, amenorrhea.

Many controversial points have arisen regarding the hormonal activity of these tumors. Certain facts are fairly well established. The estrogenic hormone of the ovary, which is the one best known, produces characteristic hyperplasia of the genital tract in the experimental animal.

After the estrogenic hormone has produced glandular hyperplasia of the endometrium, another hormone produced by the corpus luteum, called progestin (Allen [14]), prepares the endometrium for the fertilized ovum. Should fertilization fail to occur, repetition of the cycle occurs. According to the current conception, the periodic action of the estrogenic hormone and progestin is regulated by two hormones produced by the anterior lobe of the pituitary gland. These hormones of the anterior lobe are known as the follicle-stimulating hormone (prolan A) and the luteinizing hormone (prolan B) (Aschheim and Zondek [15]). The follicle-ripening hormone regulates the periodic discharge of the estrogenic hormone; the luteinizing hormone, that of progestin. It appears, therefore, that the hormone elaborated by granulosa cell and theca cell tumors is essentially of theelin character.

The second group of tumors in Meyer's classification are those which cause masculation of the woman, the arrhenoblastomas. Until the present time, about twenty-six cases of this type of tumor have been reported in the literature. Pick,[16] in 1905, first described such a tumor, which he called adenoma tubulare testiculare ovarii, and he expressed the belief that it arose from the testicular portion of an ovotestis. Meyer,

10. Löffler, E., and Priesel, A.: Beitr. z. path. Anat. u. z. allg. Path. **90**:199, 1932.
11. Melnick, P., and Kanter, A. E.: Am. J. Obst. & Gynec. **27**:41, 1934.
12. Löffler, E., and Priesel, A.: Wien. med. Wchnschr. **84**:400 (April) 1934.
13. Footnote deleted.
14. Allen, W. M.: Am. J. Physiol. **92**:174, 1930.
15. Aschheim, S., and Zondek, B.: Arch. f. Gynäk. **130**:1, 1927.
16. Pick, L.: Berl. klin. Wchnschr. **42**:502, 1905.

on the other hand, was of the opinion that the tumor originates from the sexually bivalent cells of the early embryo, that is, from embryonic rests of the germinal ridge, which differentiate in the direction of the masculine line.

In certain animals an intersexual type is known called a freemartin. This animal has the female type of external genitalia and male internal genital organs. The gonadal or sex cell is primarily female, but transition occurs from medullary cords to seminiferous tubules. In experimental work Bramwell and Parks have shown that in irradiated ovaries of fetal and very young mice the germinal epithelium proliferates and invaginates into the ovary, forming typical medullary cords as well as spermatic tubules. Thus, two types of sex reversal have been actually observed. Investigators of sex reversal have concluded that the ovary possesses a certain instability which is never observed in the testis, perhaps because in the testis the cortex becomes transformed into the tunica albuginea and therefore lacks further developmental potencies.

Three types of arrhenoblastoma have been observed by Meyer: (1) typical, well developed tubular tumors similar to the form first described by Pick; (2) atypical forms with small atypical tubules and masses of cells, and (3) a form intermediate between the two.

Clinically, these tumors occur most frequently in young adults. They cause often, but not always, various degrees of masculation. One of the early signs is deepening of the voice, and then hirsutism and atrophy of the breasts appear. Amenorrhea is always present. After removal of the tumor, the menses usually begin again. The changes in the voice regress; the growth of hair gradually ceases, and the person again reverts to a feminine habitus. These tumors are usually not malignant. Therefore, the other ovary should not be removed if the diagnosis can be made at operation, as the function of the uninvolved ovary restores normal female traits.

Because of its resemblance to testicular structures, the dysgerminoma should be mentioned. This tumor may be related to the arrhenoblastoma, but it does not have the marked hormonal influence seen in cases of the latter type. It occurs in both the ovary and the testis and is distinctly malignant. When present in the ovary it is observed in children and young adults, but when originating from the testicle it occurs most frequently in later life. In women dysgerminoma often causes atrophy of the external genitalia and the uterus, while in men it may occur in cryptorchism. These tumors are also thought to arise from cells which existed in the embryo before the stage of sexual differentiation. Dysgerminoma in males has also been called seminoma or embryonal cell carcinoma.

Another type of ovarian tumor which because of its clinical manifestations should be included with the masculating tumors is the so-called

luteoma. Bergstrand [17] reported four cases in which the tumor microscopically bore a close resemblance to hypernephroma, and because the cells were large and clear with pale-staining cytoplasm and small, oval nuclei, he suggested the possibility of their origin from luteinic cells. Clinically, the luteoma manifests itself by its masculating symptoms, simulating those of a true tumor of the adrenal.

The third group of tumors in Meyer's classification are the neoplasms which have no apparent hormonal activity, namely, the so-called Brenner tumors. These tumors have no endocrine effect, presumably owing to the fact that their origin is related to an early undifferentiated phase of development, the cells functioning along neither the male nor the female line.

Since Meyer showed that the Brenner tumor does not belong to the group of granulosa cell tumors, many cases of such tumors have been found in retrospection and in recent experience. Meyer [1] found reports of eighteen cases in a review of the literature, to which he added four of his own. In 1933 Plaut reported eight cases. Since that time many reports have appeared, chiefly in the German literature. Weinzierl [18] described one case of bilateral blastoma of the Brenner type. Fraenkel [19] reported a case as an instance of carcinoma which later proved to be a Brenner tumor. Wagner,[20] in a review of his material, observed a solid Brenner tumor. In adding the report of another case to the literature, Abraham [3] suggested that the frequency of occurrence of these tumors has not increased but that physicians have learned to recognize them. Von Szathmáry [21] reported five cases in which the tumor was present in the wall of a large cyst. Schiffmann [22] described two cases of his own and found the report of another in the literature. Examination of the illustrations in the three cases reported by Fleming [23] places the tumors in the category of the Brenner type. With the two cases described by Freund [4] and the five by Schiller,[5] it seems that a definite pathologic entity has been established. Although the main characteristics of these tumors remain constant, there are some variations which induced Meyer to subdivide them.

He distinguished two types, the first of which is the solid form, which may or may not have small and large cysts. The cells lining the cysts range from the typical large polygonal form to the type which, although similar, is pseudomucinous. The other main type is the cystoma of the ovary, in which small or large solid nodules are present in the

17. Bergstrand, Hilding: Acta path. et microbiol. Scandinav. **16**: 31, 1933.
18. Weinzierl, cited by Abraham.[3]
19. Fraenkel, L.: Zentralbl. f. Gynäk. **57**:2259, 1933.
20. Wagner, G. A., cited by Abraham.[3]
21. von Szathmáry, Z.: Arch. f. Gynäk. **154**:390, 1933.
22. Schiffmann, J.: Arch. f. Gynäk. **150**:159, 1932.
23. Fleming, Amy: J. Obst. & Gynaec. Brit. Emp. **36**:793, 1929.

wall of the cysts, which again may have epithelium characteristic of the Brenner tumor or similarly pseudomucinous epithelium.

The attachment of a name to a tumor signifies nothing in respect to its appearance or genesis. If the credit were based on historical priority, the tumor under consideration should be designated the Orthmann tumor (Plaut [2]) for in 1899, eight years before Brenner [24] reported three cases of ophoroma folliculare, Orthmann described an instance of fibroma papillare superficiale carcinomatosum ovarii dextri, which histologically, according to Plaut, was a Brenner tumor. Plaut preferred the name benign mucinous or pseudomucinous fibro-epithelioma.

In gross appearance the solid tumors and the solid nodules present in the wall of the cystomas are usually described as possessing a coarse, firm, slightly uneven surface resembling that of an ovarian fibroma. They vary from a few millimeters to the size of the head of a fetus. The tumor in the present case, as far as I can determine, is the largest on record (weighing 15 pounds [6.8 Kg.]). Neoplasms may occur on the surface of the ovary (Abraham) or in the hilus (Schiller), or they may replace the entire ovary, as in this case. They are sharply circumscribed and are yellowish; some have small and a few have large cysts in the inner portion. The tumors with a large amount of epithelium or many cysts are softer. Microscopically, they reveal dense fibrillar connective tissue, which has a tendency to become hyalinized and occasionally calcified. Everywhere, solid, ramified epithelial (epithelioid, Fleming) columns are embedded close together or far apart. Many authors designate the epithelium as indifferent. The cells are polygonal and contain usually spindle-shaped or pointed rather than oval nuclei. The cells have a tendency to swell; they then have large, clear, polygonal bodies with sharp, prominent membranes and usually resemble the cells of squamous epithelium. The cells are usually observed to contain glycogen, thus differing from the granulosa cells. They secrete colloid material, which in the larger lumens assumes a more fluid and mucinous character. Often the lumen is surrounded by flat cuboidal, low cylindric or cylindric cells. The latter type of epithelium is rare. Some cysts are lined by stratified cells and others by a single layer. The material secreted by the cells may be either mucinous or pseudomucinous (Plaut).

Since these tumors have been encountered incidentally at operation or autopsy, it is difficult to make any statement as to their frequency or age incidence. Akagi [25] reported on a tumor noted in a 9 year old girl, while Plaut described one in a woman of 73. The cases reported to the present time show that this type of tumor is three times as common in women over 40 as in those below that age and that about half of the growths have been present in women over 50 (Meyer). Except in three

24. Brenner, F.: Frankfurt. Ztschr. f. Path. **1**:150, 1907.
25. Akagi, Y.: Arch. f. Gynäk. **134**:390, 1928.

cases (those described by Weinzierl, Krompecher [26] and Maury and Schmeisser [27]), the tumor has been unilateral. As far as has been determined, the uterus undergoes no special changes. In one of the cases in the present series and in three of the twenty-two cases reported by Meyer, fibromyomas were present. In no case yet reported, to my knowledge, has any tendency been shown toward metastasis. Ewald [28] reported a case in a woman, aged 45, with carcinoma of the cervix. On the surface of one ovary a Brenner tumor occurred. Ewald pointed out the similarity of the epithelial cells in the ovarian tumor to the malignant cells of the cervix. An effect on the menses has not been observed except by Brewer and Jones [29] and by Fleming. Since the Brenner tumor has not yet been observed to have any biologic function, the interest in this type lies mainly in the histogenesis.

While one group of authors regarded the Brenner tumor as a special, more highly developed type of granulosa cell tumor or folliculoma, other investigators considered it to be a distinct entity. Only a few authors commented on the combination of solid epithelial strands and pseudomucinous epithelium and cysts. These writers did not recognize the resemblance of the tumors in their cases to the Brenner tumor. They expressed the opinion, however, that the tumors originated from foci of cells which occurred in the cortex of the ovaries of children. These cell foci were first referred to by Walthard [30] and later by Akagi. Walthard described the foci as a cluster of squamous and ciliated epithelial cells and goblet cells from which tubes and cysts were formed. Walthard pointed out that the surrounding stroma of the cortex of the ovary is rich in nuclei and favors the further growth of these foci. The ultimate fate of the foci depends on the type of surrounding connective tissue and on their growth independent of the stroma. The nearer they are to the zona vasculosa the greater is their aptitude, according to Walthard, to proliferate and lead to the formation of tumors.

The assumption that Brenner tumors develop from the cell foci described by Walthard has, therefore, much in its favor. From which cells these foci are derived is another question. It seems to be difficult to trace their first appearance during fetal development. Meyer once saw a small group of undifferentiated cells just beneath the surface of the ovary of an adult woman, and in another instance, in the hilus of the ovary. Meyer expressed the belief that these groups of cells are similar to the epithelial nodules which are often noted in and under the serosa of the tubes and ligaments. The epithelial tumors, then, originate not from the parenchyma of the ovary but from special cells which have no

26. Krompecher, E.: Ztschr. f. Geburtsh. u. Gynäk. **88**:341, 1925.
27. Maury, J. M., and Schmeisser, H. C.: Am. J. Obst. & Gynec. **27**:290, 1934.
28. Ewald, F. K.: Centralbl. f. allg. Path. u. path. Anat. **61**:81, 1934.
29. Brewer, J. I., and Jones, H. O.: Am. J. Obst. & Gynec. **25**:505, 1933.
30. Walthard, M.: Ztschr. f. Geburtsh. u. Gynäk. **49**:233, 1903.

relation to the normal development of the ovary. They owe their origin to the special ability of the surface epithelium to undergo atypical differentiation at the time when the celom epithelium forms the müllerian epithelium in the region of the wolffian body. According to Meyer, abnormal development occurs also in the region of the tubes and ligaments, where solid epithelial nodules may be formed. These epithelial nodules may be transformed into spaces lined by indifferent epithelial cells and even at times by mucous and cylindric epithelium.

Abraham pointed out that the Brenner tumors are characterized by the participation of connective tissue in their development and that this characteristic is missing in the proliferation of the surface epithelium which is observed in perisalpingitis. He also stated that while the Walthard foci are congenital maldevelopments, the proliferation of the surface epithelium appears later in life. In spite of the frequency with which epithelial nodules occur on the tubes and ligaments, tumors in these tissues are seldom observed, and a case of an extra-ovarian Brenner tumor, to my knowledge, has not been reported. The Walthard cell foci are not yet tumors, but they constitute the foundation of tumors (Abraham). It can be assumed that later, after proliferation of the epithelial cells and encapsulation by connective tissue, the cell foci become tumors. Abraham expressed the belief that the cellular proliferation cannot be designated a neoplasm unless it is separated from the surrounding tissue by a capsule. In the first case in my series the tumor was so large that normal ovarian tissue could not be recognized.

Since the Walthard cell foci are embryonal rests, has one, therefore, to assume that the formation of the Brenner tumor and other tumors the cells of which resemble histologically the Walthard type has been determined during embryonic life? Plaut expressed the belief that this was not true. Waldeyer [31] suggested that in later life the surface epithelium can proliferate. In adults it has repeatedly been observed that the surface epithelium of the ovary and, in general, the peritoneal epithelium of the female pelvis can proliferate. Plaut expressed the belief that single cells or groups of cells, especially under the influence of the hormones and the circulatory changes of the female genitalia, may not remain unchanged from the time of embryonic development. Goodall,[32] however, maintained that embryonic rests in the ovary, like similar rests in other parts of the body, may remain dormant for all time or may be awakened into activity by any one of many stimulants at any age of the host. Many, if not all, of these rests are aroused to proliferation as a result of nutritional changes. These may be primary vascular changes or neutrotrophic. The nutritional influence may

31. Waldeyer, cited by Meyer.[1]

32. Goodall, J. R., in Curtis, A. H.: Obstetrics and Gynecology, Philadelphia, W. B. Saunders Company, 1933, vol. 2.

operate by stimulation of the normal ovarian function to abnormal development or by stimulation of the abnormal embryonic rests in the ovary to pathologic activity and sometimes to unbridled aberrant growth.

Theoretically, there is the possibility that single epithelial strands of the Brenner type appear in fibromas of the ovary and that the Brenner tumor takes its origin from such a source. Plaut made serial sections of a fibroma which occurred in the wall of an ovarian cyst and obtained no evidence of epithelial elements. Also, in one of his cases in which a Brenner tumor occurred on one side and a fibroma of the ovary on the other, there was no evidence of epithelial elements on examination of several blocks.

Aside from the origin of the Brenner tumor from the cell foci described by Walthard, Schiller suggested another possibility. He reported seven cases and expressed the belief that the Brenner tumor may have its origin in the rete ovarii. According to Fischel,[33] both the rete and the medullary cords and even the granulosa arise not as derivatives of the so-called germ epithelium but as local differentiations of the ovarian mesenchyma. If the rete, medullary tubes and epoophoron arise from a local condensation of the mesenchyme, it seems easier to explain the transition of one structure into the other and assume that in the original parenchyma there are abnormal potentialities. Schiller pointed out that the epoophoron may not lose its connection with the rete and that the wolffian duct has the potentialities seen in the cells of the Brenner tumor. He stated that the formation of the fibrotic mantle about the epithelial cells is secondary and reactive. The origin of Brenner tumors from the rete and the tubes of the epoophoron, which Fleming suggested on theoretical grounds, does not speak against their origin from the surface epithelium. The surface epithelium is derived from the primary mesoderm and belongs to the connective tissue of the celom, as does the parenchyma of the sex glands. It has been shown by Meyer, Schiffmann, Steiner [34] and others that the celom epithelium of the ovary has the ability to form undifferentiated squamous epithelium like that seen in the peritoneum of the tube and the broad ligament. The ability of the ovarian epithelium to develop into mucus-producing epithelium does not change in childhood or later after the climacteric. The potentiality remains. The appearance of granulosa cell tumors in old women, twenty years after the climacterium, indicates that fetal cells may retain their developmental potency after being dormant for decades and may suddenly display their power of differentiation and proliferation. According to Schiller, one can therefore assume two possibilities of origin for the Brenner tumor, namely, the surface epi-

33. Fischel, Alfred: Grundriss der Entwicklung des Menschen, Berlin, Julius Springer, 1932.

34. Steiner, H.: Zentralbl. f. Gynäk. **50**:258, 1926.

thelium of the ovary and the urogenital anlage. This double origin finds its analog in the case of pseudomucinous cysts, which may originate from the downgrowth of surface epithelium or from the entodermal parts of teratomas resembling intestinal tissue.

If one agrees with Fischel's conception of the origin of the ovarian constituents, one can consider the tumors of the ovary already described as having a common derivation, namely, from a local condensation of mesenchyme. The stage of arrest in the differentiation of these cells or the dominance of one of the original potentialities over the other leads to the characteristics shown by the fully developed tumor. The least differentiated of all, then, is the Brenner tumor, which has not developed to such an extent as to present masculating or feminizing traits in the host. The sex is determined by the chromosomal variations of the germ cells, more particularly those of the spermatozoon. The presence of the single x-chromosome, on the one hand, or of the two x-chromosomes, on the other, apparently determines the male or the female direction of zygotic development. In addition to this, biologists are coming to believe that the balance between the sex chromosomes and the autosomes is of great importance as well. The primary sex-differentiating force originates from the zygote produced by the union of the male and female germ cells or gametes. In the higher forms, however, this force can be modified or overridden by the endocrine influence of the gonads and other affiliated glands. It should be added that the zygotic influence is believed to determine whether the gonad is to be an ovary or a testis, so that, in the final analysis, a single motivating force is behind the whole process of differentiation. In abnormal developments, however, the hormonal production of the gland is either suppressed or overshadowed by the secretion of the tumor. If the motivating force in the undifferentiated tumor cell is predominantly masculine, arrhenoblastoma results. If the feminizing element is predominant, development of a granulosa cell or theca cell tumor results. Finally, if the motivating force is in neither direction, a mucinous or pseudomucinous fibro-epithelioma results.

SUMMARY

Three cases of benign fibro-epithelioma of the ovary (Brenner tumor) are described. Two of the tumors were discovered incidentally at autopsy. The third tumor, of unusual size, was removed surgically. Since little discussion of the histogenesis of this tumor occurs in the English literature, the current views of its origin are presented.

In accordance with the conception of Fischel, it is suggested that these tumors as well as the masculating arrhenoblastomas and the feminizing granulosa cell and theca cell tumors have a common stem of origin and merely differentiate along different lines.

EXPERIMENTAL THROMBOPENIC PURPURA IN THE DOG

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Thrombopenic purpura as produced experimentally with antiplatelet serum in the dog resembles hemorrhagic purpura in man so closely that it affords an excellent means of studying the related problems of this disease. A physiopathologic study of the disturbances that accompany the destruction of platelets or a reduction in their number may throw much light on the rôle that they play in hemostasis, vascular permeability and repair and metabolism and afford a glimpse into the type and approximate magnitude of the forces disturbed by changes in this element. The dog was selected for the experiments because of the advantages that it offers over the smaller laboratory animals in this type of study. If care is exercised in preparing the serum and choosing the dose and route of administration, the dog nearly always recovers from an acute attack. In this report are presented observations on the clinical course and associated changes in a group of sixty-three dogs in each one of which purpura was produced one or more times.

Thrombopenic purpura has been produced in animals experimentally with benzene, diphtheria toxin,[1] pneumococcus extract [2] and thorium.[3] It has been brought about in guinea-pigs, rabbits and rats by means of antiplatelet serum by several workers interested in the origin and functions of the blood platelet [4] and in the pathogenesis of the hemorrhages.[5] However, there are many agents capable of producing thrombopenia in an animal without being purpurigenic. Such are, for example, the

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1. Duke, W. W.: J. Exper. Med. **14**:265, 1911.

2. Reimann, H. E., and Julianelle, L. A.: J. Exper. Med. **43**:87, 1926.

3. Shih, H. E., and Jung, T. S.: Proc. Soc. Exper. Biol. & Med. **29**:243, 1931.

4. Ledingham, J. C. G., and Bedson, S. P.: Lancet **1**:311, 1915. Gottlieb, M. J.: J. Immunol. **4**:309, 1919. Lee, R. E., and Robertson, O. H.: J. M. Research **33**:323, 1916. LeSourd, L., and Pagniez, P.: Compt. rend. Soc. de biol. **58**:109 and 562, 1906.

5. (*a*) Watabiki, T.: Kitasato Arch. Exper. Med. **1**:195, 1917. (*b*) Roskam, J.: Sang, **8**:129, 1934. (*c*) Bedson, S. P.: J. Path. & Bact. **25**:94, 1922.

roentgen rays,[6] saponin,[7] electrargol (a colloidal suspension of silver), colloidal arsenic sulfide, egg albumin, lecithin, acacia, peptone and some vegetable and animal oils.[8] An injury to the blood vessels, as well as thrombopenia, seems necessary for the production of the disease.[5b, c]

MATERIAL AND METHODS

Material.—Mongrel breeds of foxhounds, beagles and basset-hounds, collies and wire-haired and bull terriers, between 8 and 20 Kg. in weight and from 1 to 2 years of age, were used in these experiments. Each animal was housed in a metabolism cage and fed a diet consisting of regular amounts of compressed food biscuits and fresh meat, with a free supply of water. Feeding took place at the end of the day, after all the determinations had been performed. A few animals were raised in the institution, and none became an experimental subject until after a preliminary period of training to lie on the table without struggle or excitement. During the first and second days of the disease the animal was under practically continuous observation; later, observations were made one or more times a day, depending on the stage of the disease.

Preparation of the Serum.—Between 110 and 120 cc. of dog's blood was collected by cardiac puncture into chilled syringes containing an excess of a cold 3 per cent solution of sodium citrate in physiologic solution of sodium chloride (two parts of the solution for one part of blood). The platelets were isolated by fractional centrifugation, washed three times in saline solution or until no red or white blood cells could be detected microscopically and injected intravenously into a rabbit, this process being repeated six times at five day intervals. Ten days after the last injection the serum was collected, heated to 56 C., filtered through a Seitz filter and stored in ampules. Because of a slight loss in potency from aging, the serum was never used later than four months after its preparation and titration.

Titration of the Serum.—The agglutinating titer of the serum, as determined with the technic and precautions described by Watabiki[5a] and Bedson,[5c] ranged between 1:80 and 1:320. An idea of the purpurigenic properties of the serum, however, could not be gained from its platelet-agglutinating titer in vitro. The same has been found true regarding qualities of other cytotoxic serums.[9] The fact that a drop of the serum when injected intradermally produces a hemorrhagic patch proved to be useful as a measure of its potency. Various dilutions (from 1:2 and 1:4 to 1:128 and 1:256) of the serum were made in physiologic solution of sodium chloride, and 0.05 cc. of each was injected intradermally into separate areas in the abdomen of a dog. Four sets of inoculations were made each time, one on each side of the abdominal skin of each of two animals that had not previously received any serum, and the results were read at the end of twenty-four hours. The purpurigenic titer of the serum was expressed in terms of the highest dilution that brought about a hemorrhagic patch in at least two of the four inoculations. Serum with a titer lower than 1:32 or higher than 1:64 was not used.

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6. Leschke, E., and Wittkower, E.: Ztschr. f. klin. Med. **102**:649, 1926.

7. Firket, J.: Compt. rend. Soc. de biol. **85**:730, 1921. Bunting, C. K.: J. Exper. Med. **11**:541, 1909.

8. Aynaud, M.: Le globulin des mammiferes, Thèse de Paris, 1909.

9. Hueper, W. C.: Arch. Path. **13**:584, 1932.

Dosage and Methods of Administration.—One-tenth cubic centimeter of antiplatelet serum of the already mentioned titer per kilogram of body weight, injected intraperitoneally, was sufficient to produce thrombopenia lasting approximately forty-eight hours in animals of the age and breeds named. Such a dose will be referred to as moderate and was the one used most frequently intravenously and intraperitoneally. A serum of low titer may, when injected intradermally in full strength, bring out a hemorrhagic patch, but larger doses will be necessary to produce the required effect, and therefore such serum was not used in comparative experiments. The intraperitoneal and intravenous routes were those most commonly used. The serum was also injected subcutaneously and intradermally and instilled into various orifices and sacs.

RESULTS

Effect of a Single Moderate Dose.—Most animals showed no signs of distress immediately after an intraperitoneal injection of the serum but from ten to fifteen minutes afterward usually vomited or passed a stool. They remained quiet for the next hour and then gradually regained their alertness and former appetite. Throughout the acute period of purpura they showed little alteration in their behavior, and were it not for the changes in the blood and the hemorrhages one could not differentiate them, at a glance, from normal dogs. Usually from four to five hours after an intraperitoneal injection the first signs of an increased tendency to bleed appeared, that is, a slight prolongation of the mean bleeding time, a positive petechial reaction in the skin and a slight reddening about the margins of any previous cuts or abrasions. This reddening gradually became wider and more intense and later changed to a deep purple.

A slight rise in the rectal temperature was generally observed. The rise was first detected approximately from four to five hours after an intraperitoneal injection and was present for the next twenty-four to forty-eight hours. It varied between 39 and 40 C. (102.2 and 104 F.) and never rose above 41 C. (105.8 F.) in any animal. The pulse and respiration rates showed no significant changes except during the first few minutes after the injection. During the acute phase the animals often shook violently with chills.

Twenty-one dogs received a single moderate dose intraperitoneally. With one exception they all survived. The average number of days that the attack of purpura lasted was two and seven-tenths; two dogs had it for one day only and one dog for six days. During the attacks the testis and the shaft and prepuce of the penis in the male and the mammary glands in the female invariably showed hemorrhages. Elsewhere, hemorrhages and purpuric patches appeared in the following locations in the order of frequency: on the external ears, the conjunctiva, the nipples and the oral mucous membrane, about the margins of the gums, in the axilla, on the inferior and lateral surfaces of the

tongue, on the tonsils and soft palate, at the base of the nails, in the anterior ocular chamber and over the iris. Epistaxis to the point of occlusion of both nares with blood clots appeared in several animals. Hematemesis was rarely observed, but rectal bleeding and tarry stools were common; vaginal bleeding and hematuria were noted several times and occasionally prolapse of the rectum. Scarred areas of the skin usually became hemorrhagic.

The skin became softer than usual, pitted on pressure and appeared to have lost some of its tonicity. When a fold was lifted between the fingers, held for a time and then released, it sank back very slowly and remained wrinkled for a much longer period than before the serum was administered. An example of the method of investigating this response and its results in one animal follows: A line 20 mm. long was drawn with ink on the skin of the abdomen and then stretched until it measured 30 mm., by picking up the skin at either end, pulling it in opposite directions and holding it thus for a period of thirty seconds. Immediately after release and afterward at thirty second intervals the line was measured and its length recorded.

Dog 49 (Normal Female Foxhound).—Two individual lines, 20 mm. long, were drawn on separate areas across the abdominal skin. The length in millimeters immediately after release and at thirty second intervals was: First line, 22, 21.5, 21, 20; second line, 22.5, 21.8, 21, 20.5, 20. During an attack of purpura the first line measured 24, 24, 23.2, 23.1, 23, 23, 23, 22.8, 22.6, 22.5, 22.5, 22.4, 22, 22, 21.2, 21, 20.7 and 20.5 mm.

To this loss of tonicity was attributed the wide gaping of cuts in the skin, and, perhaps as a result of it and the failure of the skin to be in close apposition to underlying tissues, the blood surged out of the wound and spread rapidly under the skin in all directions. The rapid infiltration of cuts with blood during this period was in sharp contrast with the slower development of the same phenomenon during the early part of the attack. After the hemorrhagic period was over and the number of platelets had increased above normal levels the skin became unusually tense and difficult to stretch to the required length. When released it returned to its previous state at once or within an interval of thirty seconds. Areas that had been cut or traumatized as early as from three to four days before an injection of the serum were favorite sites for the development of hemorrhages.

Dog 7 (Normal Female Collie).—Daily for six days a small cut, from 4 to 5 mm. long and from 2 to 3 mm. deep, was made in the skin of the abdomen, care being taken to keep the cuts 2 cm. apart and to plot their relative positions on a chart. Twenty-four hours after the sixth cut had been made the animal was given one dose of antiplatelet serum intraperitoneally. Hemorrhagic infiltrations appeared about the cuts made one, two, three and four days before the injection.

During the acute stage the skin often became dusky or assumed a light violet tinge. Alternate lividity and redness of the untouched skin and oral mucous membrane followed one another in rapid succession. Ordinary handling of the ears caused a dusky erythema to appear, which lasted for several minutes, the ears being covered the next day by hemorrhages of various sizes. The appearance of petechiae after stroking of the skin of the abdomen of the animal was one of the earliest signs of an increased tendency to bleed. From three to four hours after an intraperitoneal injection this maneuver resulted in the appearance of bright red or, in some cases, purple petechiae, of various sizes. The response was more marked and prompt when the animal was at the height of the acute stage than in the beginning of it. Strongly positive reactions were obtained as early as two minutes after an intravenous injection of the serum. The skin of any portion of the body responded in the same fashion but in various degrees, that of the ears being particularly susceptible to the trauma. Petechiae could be brought out by the same method in the oral mucous membrane. The light application of a tourniquet to an extremity for a period of one minute likewise was followed by a crop of petechiae, which often lined themselves in a row along the course of veins.

The blood emerging from cuts often assumed a color similar to that of juice from dark grapes, and perhaps on this account the petechiae and other extravasations of blood brought about by traumatizing the skin were of the same color. Toward the end of the hemorrhagic period the blood from the vein of the ear often appeared "chalky," and large globules of fat floated on its surface; when extravasated over a rag or a piece of absorbent paper it seldom dried within the usual period. In one instance a cloth was still wet twenty-four hours after being soaked with blood from an animal with acute purpura, although the room temperature was 70 F. and the air dry. Clots from blood obtained during this stage did not dry when removed from the vessel and exposed to the air for a considerably longer period than clots from the blood of normal dogs. In a few instances after the clot formed an accumulation of a material resembling chicken fat was observed. The material could be precipitated with the proteins by treatment with ammonium sulfate; it could not be extracted with the ordinary fat solvents (alcohol-ether mixture) and did not stain black with osmic acid. Determinations of plasma protein and cholesterol showed only insignificant departures from the normal range. Heating at various temperatures ranging from 40 to 90 C. and finally boiling vigorously had no effect on its gross appearance. Cooling to 0 C. did not change it grossly. Microscopically, the material consisted of a great number of minute colloidal particles in extremely active brownian movement and a few globules of fat.

It was observed nearly always about thirty-six hours after the intraperitoneal, and from eighteen to twenty-four hours after the intravenous, injection of a moderate dose and may be a manifestation of the hyperlipemia known to follow repeated bleedings.[10] The actively moving particles resembled in appearance the kinetocytes[11] that have been described as being present in increased number in purpura. Spontaneous precipitation from the blood of a protein substance has been observed in a human being with multiple myeloma, thrombopenia, circulatory disturbances and a prolonged bleeding time.[12]

From four to six days after an animal had fully recovered from an attack no signs of any hemorrhage or reddening about cuts of the skin could be detected; the hemorrhages in the oral mucous membrane and the conjunctiva disappeared, and no other external evidence of the attack was discernible. There are now in the colony several animals living in good health two and one-half years after their last attack of purpura; in one of these purpura was produced seven times. Five animals became pregnant subsequently and gave birth to healthy pups that grew to maturity. One pregnant animal was given the serum four times at three day intervals and had a very severe attack of purpura, but one week after the last injection it gave birth to eleven healthy puppies. Their platelet counts did not differ from those of other puppies of the same age, and they showed no tendency to purpura. Two of these puppies were given a small dose of antiplatelet serum and contracted purpura, although the mother had already become refractory to the serum before giving birth to them. The serum was equally effective when given to two 3 year old animals.

Effects of Massive Doses.—Eleven animals were given single massive doses intraperitoneally (1 cc. per kilogram of body weight). Three died less than twelve hours after the injection; one died five and another six days after, and the remaining six survived after long periods of thrombopenia, lasting in two instances seventeen days. The animals were usually prostrated by the injection; those that survived it remained listless and refused food for several days. The purpura lasted for a variable length of time, in two instances eleven days. One dog recovered after a drop in the erythrocyte count from 7,100,000 to 950,000. Another lived for five days after the injection but presented widespread hemorrhages, marked edema of the extremities and severe anemia. The animals that lost a good deal of blood became dyspneic, walked uncertainly, swaying about, and fell frequently as though in a faint. In

10. Bloor, W. R.: J. Biol. Chem. **49**:201, 1921. Leiter, L.: Arch. Int. Med. **48**:1, 1931.

11. Edelmann, A.: Wien. klin. Wchnschr. **44**:795, 1931.

12. Wintrobe, M. M.: Bull. Johns Hopkins Hosp. **52**:156, 1933.

those that survived the injection only a few days the hemorrhages of the skin failed to clear up in the center, as they usually do in animals recovering promptly from an attack. Death appeared to follow extensive loss of blood (mostly in abdominal and thoracic hemorrhages) and visceral damage. External cutaneous manifestations seldom gave any indication of the extent and degree of the visceral involvement. The pathologic changes in the organs of animals that succumbed to injections of the serum, as well as of those that received moderate doses and were killed at various intervals afterward, are the object of a separate study.

Effects of Repeated Moderate Doses.—Repeated injections of the serum conferred a resistance against its purpura-producing qualities. Eight dogs were given a series of repeated intraperitoneal injections of moderate doses of antiplatelet serum at intervals of from three to four days. A resistance against the purpurigenic qualities of the serum developed in all. The average number of moderate doses needed was three and six-tenths, and the average total amount of serum was 7.3 cc. The attacks that followed the first and second doses were the most severe. A single dose sufficed to render one dog refractory to subsequent doses; another dog required seven doses to reach the same stage. An animal was considered to have become refractory when the twice-repeated injection of a dose that had previously caused purpura failed in both instances to bring it about. This refractory stage has been observed to develop in guinea-pigs, rabbits and rats that have had one or more doses of antiplatelet serum [5a, c] and in mice rendered purpuric by injections of pneumococcus extract.[13] It has been ascribed by some to the development of an antibody against the platelet antibody of the antiplatelet serum [5a] or to a precipitin for the particular serum carrying the platelet antibody.[5c] Bedson found that the serum of normal rabbits alone was capable of conferring this resistance on animals that later received antiplatelet serum made in rabbits. If this were true, an animal that had become refractory to the purpura-producing qualities of an antiplatelet serum prepared in the rabbit would not react in this way to a similar serum made in an animal of another species.

An antidog-platelet serum was prepared in a goat. Eighteen intraperitoneal inoculations, each of the platelets in from 110 to 120 cc. of dogs' blood, were necessary to bring the serum to an intradermal purpurigenic titer of 1:32. Eleven dogs were given variable amounts of antiplatelet serum until they became refractory to its purpura-producing qualities. Eight received the injections of antidog-platelet rabbit serum first and two, three, six, nine, eighteen, nineteen, twenty-eight and twenty-nine days, respectively, after the last injections were given two or more doses of antidog-platelet goat serum. For the remaining three

13. Julianelle, L. A., and Reimann, H.: J. Exper. Med. **45**:609, 1927.

of the group this procedure was reversed, the goat serum being given first. In every instance purpura developed as a result of the initial injection of the second series. The severity of the second attacks varied but not to any greater degree than could not be accounted for by the individual differences already noted between the animals.

Local Effects of the Serum.—The cytotoxic properties of the serum were well demonstrated when it was injected subcutaneously. Great swelling and discoloration appeared at the site of injection within two hours; the tissues about it became edematous, and sometimes sloughing and ulceration followed. If enough of the serum was injected, thrombopenia and generalized purpura resulted. The intradermal injection of a drop of the serum brought out a sharp, well defined purpuric patch within from three to four hours, which remained for from twenty-four to forty-eight hours. Placing the serum in an open wound or over the oral mucous membrane, instilling it into the nares or into the conjunctival sac and giving it by mouth did not give rise to any local or general hemorrhagic manifestations. This was perhaps because not enough time was allowed for the serum to act on the same tissue area.

Anaphylactic Phenomena.—There were mild anaphylactic phenomena when injections of the serum were repeated from five days to as late as four and a half months after the preceding injection. If the injections of serum were spaced not more than four days apart, only mild signs of anaphylaxis were noted. In the entire series only two animals died with signs of anaphylactic shock, one immediately after, and the other within twenty-four hours of, a second injection. In one of the fatal cases a period of nine days had elapsed since the preceding injection; in the other thirty-two days had elapsed. The mean bleeding time was short, and the petechial reaction of the skin was negative in both instances during the shock. Autopsy yielded the characteristic observations of anaphylactic shock.

COMMENT

As is natural to expect, dogs vary in their reaction to a uniform dose of antiplatelet serum of standard strength, making it difficult to establish a lethal dose. A serum of an intradermal titer of from 1:32 to 1:64 may, however, be given intraperitoneally or intravenously in doses of from 0.08 to 0.1 cc. per kilogram of body weight to dogs of the type indicated with reasonable assurance that the animal will have an attack lasting from two to three days and recover spontaneously. Doses above 0.5 cc. are often fatal. Serum administered intravenously appeared to be better tolerated than that given intraperitoneally or subcutaneously. The opportunity for prolonged contact in strong concen-

tration with the tissues and the consequent extensive local hemorrhages and edema perhaps aggravated the other effects. Serum injected intravenously was rapidly neutralized and diluted, and its effects, although more rapid and widespread, should be less intense per unit of area affected. In most respects the features of the disease were identical with those observed in a large series of human patients.[14] As with human beings, there was frequent spontaneous recovery from attacks, even after large amounts of blood had been lost and severe anemia prevailed.

The refractory state to a purpurigenic dose of antiplatelet serum that appears after an animal has received one or more doses of the serum seems to depend not on any newly acquired ability of the animal to combat purpura per se but rather on an immunity that has developed against a certain purpurigenic agent, for it ceases to operate when the nature of the agent is changed. The resistance to purpura that develops in mice and rabbits after injections of pneumococcus extract is probably of a different nature, since it was shown [13] that the purpura-producing principle in the extract does not stimulate the production of demonstrable antibodies and that the serum of immunized animals does not give protection against it. It would be interesting to determine whether or not mice made refractory to purpura-producing doses of pneumococcus extract would acquire purpura after the injection of antiplatelet serum.

The changes in the skin suggest that an upset of the usual quantitative relationships between venule or capillary blood pressure and tissue pressure may play a part in producing the edema and the prolonged bleeding time. A decrease in elasticity of the skin and in tissue pressure have been observed clinically in edema,[15] the decrease in elasticity appearing before the edema could be demonstrated by pitting. Accumulations of fluid and hemorrhages take place more frequently and in greater degree in body cavities and soft, yielding tissues. In this state the skin would conceivably offer less resistance to the escape of fluid as well as of blood from neighboring vessels. The strong petechial reaction elicited during the acute period is perhaps also influenced by this loss in tonicity. The "easy bruising" so often met with in patients with purpura may likewise be favored by the little support offered by the skin to the vessels when trauma is received.

During a phase of purpura hemorrhages developed more frequently in areas that had been injured recently. This may explain the spontaneous hemorrhages of patients with thrombopenia. The trauma to which they were subjected and of which they have no recollection might have been light and preceded an attack of thrombopenia by several days.

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14. Jones, H. W., and Tocantins, L. M.: J. A. M. A. **100**:83, 1933.

15. Schwartz, A. B.: Arch. Int. Med. **17**:398, 1916. Holland, G., and Meyer, F.: Arch. f. exper. Path. u. Pharmakol. **168**:603, 1932.

But for the onset of that attack the effects of the trauma (the cutaneous hemorrhage) would not have been visible or felt.

Freund [16] has advanced the view that the hemorrhages in the cutaneous lesions of guinea-pigs following intravascular injections of toxins (Schwartzman phenomenon) may be simply the result of the augmentation of what would have been only a transient injury. Likewise, in our animals the trauma applied to the skin (abrasion, cut) would ordinarily have healed promptly and left no trace. The subsequent injection of antiplatelet serum, either by disrupting the mechanism of repair of traumatized tissues or perhaps by injuring them further, allowed the development of hemorrhages in those previously injured areas.

SUMMARY

Thrombopenic purpura, in most respects resembling its equivalent in man, can be produced experimentally in the dog with an antiplatelet serum of approximately uniform potency. Moderate doses of the serum produce an attack lasting approximately forty-eight hours, followed by a spontaneous recovery. Large doses are often fatal, death apparently resulting from massive hemorrhages. Repeated injections at close intervals render an animal refractory to the purpurigenic qualities of the serum. This refractoriness appears to be simply the result of immunity built up against the particular serum carrying the platelet antibody, for it does not operate against an antiplatelet serum prepared in an animal of a different species. During the acute stage of purpura marked physical changes take place in the skin and blood that may be directly related to the pathogenesis of the hemorrhages.

16. Freund, J.: J. Exper. Med. **60**:661, 1934.

PRIMARY TUBERCULOUS INFECTION OF THE INTESTINE

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The discovery of the bovine tubercle bacillus by Theobald Smith in 1896 and the statement by von Behring in 1903 that a large percentage of all cases of tuberculosis originate in childhood by infection through the intestinal tract inaugurated a controversy. It is now generally agreed that the bovine tubercle bacillus is virulent for man and that clinical tuberculosis may follow a primary infection of the intestinal tract (ingestion form of primary tuberculous infection). The exact percentage of primary infection which occurs by this route is, however, still a matter of dispute. An undoubted case of primary tuberculous infection of the intestine is presented herewith, together with a review of the literature in an attempt to determine the frequency of indisputable cases.

REPORT OF A CASE

A white boy died at the age of 8 years. At the age of 2 years he spent three months in the country, where he received fresh milk from cows which had not been inspected. He visited the same place somewhat later in the same year. At the age of 3 years he spent some time at another resort where, however, he received milk from inspected cows.

Autopsy was made by Dr. K. Leslie Stout twelve hours after death. There was no free fluid in the peritoneal cavity. The peritoneal surfaces were smooth and glistening. The omentum was free. There were no unusual masses except in the mesentery adjacent to the cecum and terminal ileum. Here there was a cluster of lymph nodes which were enlarged and matted together. The pleural cavities contained no abnormal fluid. The surfaces were smooth and glistening. The lungs were partly collapsed.

The pleural surfaces of the lungs were smooth, and there was no fibrin or exudate present. Along the posterior margins there were small coalescent areas which were slightly depressed and had a blue tinge. Palpation of both lungs revealed irregular areas of consolidation throughout all lobes. On cut section the surfaces were irregularly mottled and showed many pinpoint-sized grayish-yellow dots which sometimes appeared in clusters. The tracheobronchial lymph nodes were carefully examined, were not unusually prominent, were soft and on section showed no abnormal architecture. The lungs were examined most carefully, both by multiple incisions and by making roentgenograms, for a lesion which might be considered as a primary site of tuberculous infection. The lymph nodes in the thorax were sectioned, and the nodes in the neck palpated, and were not found to be abnormal.

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The heart weighed 140 Gm. and showed no abnormality. The spleen weighed 75 Gm. Through the transparent capsule a few minute grayish-yellow areas could be seen. The consistency of the organ was not altered. The cut surface showed a light bluish-red surface in which the malpighian corpuscles appeared to be of average size and frequency. The kidneys showed fetal lobulation. There were no gross abnormalities. The aorta, liver, pancreas, adrenals, ureters, bladder, prostate and seminal vesicles macroscopically appeared to be normal.

The mucosa of the esophagus showed postmortem softening. In the terminal ileum the lymphoid tissue was somewhat hyperplastic. The vessels in the vicinity were injected. No ulceration or hemorrhages were found. The rest of the stomach and the bowel showed no abnormalities.

In the angle between the terminal ileum and the cecum there was a cluster of nodes, the largest portion of which measured as much as 4 by 2 by 1.5 cm. The nodes were matted together and varied in consistency. Section showed irregular areas of grayish-yellow coagulation necrosis with adhesions of the capsules of adjacent nodes. The nodes along the iliac arteries, along the aorta, at the root of the mesentery and at the celiac axis were examined macroscopically and not found to be abnormal.

By cisternal puncture 20 cc. of a dark brownish-black spinal fluid was removed. The brain weighed 1,500 Gm. There was considerable flattening of the cerebral convolutions, and the sulci were correspondingly diminished. The cerebrospinal fluid was found chiefly at the base and laterally. The surface of the cerebral hemispheres appeared to be dry. The pia arachnoid of the cerebral hemispheres was dull and granular. In the lateral fissures at the base and over the frontal, parietal and temporal lobes there was a grayish-yellow exudate. This was particularly abundant over the base and gave a grayish-yellow edematous appearance to the pia arachnoid in this region. After fixation in solution of formaldehyde it was seen that the granularity of the pia arachnoid was due to myriads of pinpoint-sized gray dots. The ventricular system did not show anything unusual except for the ependyma, which appeared dull and beset with the same granules which were found in the pia arachnoid. The pia arachnoid of the spinal cord was edematous and dull and showed patches of exudate, which was most marked over the dorsal aspect. Serial sections of the brain, fixed in solution of formaldehyde, revealed no tumor masses, in particular no tuberculomas.

Microscopic Observations.—Only the organs showing abnormalities of importance will be described.

The spleen revealed hyperplasia of its pulp. Only one tubercle, but this of characteristic structure, was found in the sections.

A section of the terminal ileum was taken to include a portion of Peyer's patches which macroscopically appeared to be hyperplastic. Many miliary tubercles were found in the lymphoid tissue of the mucosa. They were of characteristic structure, and some showed central caseous necrosis. At one point a subserosal tubercle was discovered which appeared to be surrounded by a fibrous wall. The central portion of this tubercle had undergone necrosis.

No abnormalities were found in the cecum.

The entire architecture of the mesenteric lymph nodes was obliterated by fairly extensive irregular areas of coagulation necrosis and by a number of typical miliary and conglomerate tubercles. There was abundant connective tissue proliferation in the periphery of the tubercles, in which there was a scattering of lymphoid cells and many giant cells of the typical Langhans type.

The pia arachnoid of the brain was more or less diffusely infiltrated by lymphocytes and monocytes. Here and there a typical tubercle could be seen. The

exudate was more abundant in the sections taken of the pons, and here the tubercles were more definite and showed more central caseous necrosis.

In the lungs foci were found in which the alveoli were filled with a cellular exudate. These foci tended to confluence. The exudate consisted principally of polymorphonuclear leukocytes and round cells. There were a few phagocytes containing pigment. These areas of cellular exudation were found chiefly around bronchioles, and in such cases the bronchioles were packed with a purulent exudate. The involvement of the bronchioles was striking. In about six sections there was only one which showed the characteristics of a tubercle.

Bacteriologic Report (E. E. Ecker).—Sediment obtained by centrifugating spinal fluid showed acid-fast bacilli. A smear from a mesenteric lymph node and one from the left lateral fissure of the brain showed acid-fast bacilli. Attempts at cultivation of organisms from lymph nodes and spinal fluid gave practically invisible growth on glycerin agar, potato and glycerol broth. Smears showed short and acid-fast rods. The organism was evidently dysgonic. Transplantations were made on numerous mediums: Santon's, Corper's, Petraganini's, Löwenstein's and Petroff's. Similar attempts at cultivation were made by Dr. Franklin Miller. He secured a pinpoint-sized colony, unpigmented, on a simple inspissated egg medium, which failed to continue to grow. The culture which I secured showed the same dysgonic behavior, and growth could not be obtained in sufficient quantities for inoculations into rabbits. The dysgonic behavior, unpigmented character and sparse growth indicate that the organism was possibly of bovine origin.

Final Anatomic Diagnosis.—The diagnosis was: tuberculous cerebrospinal meningitis; miliary tuberculosis of the lungs and of the spleen; primary tuberculous infection of the terminal ileum with massive caseous necrosis of the adjacent mesenteric lymph nodes; bronchopneumonia; bullous pulmonary emphysema; interlobar, on the left side; slight fatty metamorphosis of the liver; acute purulent conjunctivitis, bilateral; corneal ulceration, and emaciation.

Summary of Observations at Autopsy.—A miliary tuberculosis was present which in the gross was most evident in the meninges. A careful search of the lungs for a primary infection revealed none. On the other hand, the lesion in the ileum appeared to fulfil the requirements of such a lesion. The organism, undoubtedly Mycobacterium tuberculosis, was probably of the bovine variety, although unfortunately its inoculation into animals could not be obtained. Ingestion of milk from an uncontrolled source five years previous to death cannot have been the cause of this infection since at autopsy there was found an early primary focus.

A search through the records of the pathologic laboratory of the City Hospital, where a relatively large number of cases of tuberculosis come to autopsy, revealed only 2 other cases in the past two years which might be considered as examples of primary intestinal tuberculosis.[1]

1. A continuation of this study has revealed 5 more cases of primary tuberculous infection of the intestine, all obsolete. In 3 of these the intestinal infection was associated with a pulmonary primary complex. The relationship of cases of primary intestinal to cases of pulmonary infection in this group is that of 1: 17.3. The percentage of cases of primary intestinal infection in the total group of 592 routine autopsies on persons of all ages is about 1; that of cases of demonstrated primary pulmonary infection is 20.

REPORT OF PROBABLE CASES

A white boy, aged 9 years, was admitted to the City Hospital because of anemia, the cause of which was not discovered. The child had a positive tuberculin test, but otherwise no signs or symptoms of clinical tuberculosis. Bronchopneumonia was the immediate cause of death.

The autopsy (Mae Gallavan) was made ten hours after death. The abdomen contained no free fluid. The surfaces were moist. There were no adhesions, no abnormal masses. The left pleural cavity contained 100 cc. of clear fluid. There were no adhesions in either cavity.

The pleural surfaces of the lungs were smooth and glistening. Nodular areas were palpated throughout all lobes. On section the right upper lobe cut with the consistency of spleen. The surface was finely granular. Much of the same picture was seen in the other lobes. The lymph nodes were examined carefully. They were all hyperplastic and brownish-red mottled with black, and on section the surface bulged. In the left upper bronchopulmonary node small soft yellow areas were found. This node measured 1 cm. in its greatest diameter. The rest of the nodes showed no such change. Subsequent to fixation the lungs were more carefully examined by multiple section and fluoroscopy, but aside from the lesion described in the left upper bronchopulmonary node no evidence of tuberculosis, either recent or remote, was discovered.

The mesenteric lymph nodes were all large, measuring up to 2 cm. in diameter. They were yellowish pink. A lymph node found in the angle between the terminal ileum and the cecum measured 7 mm. in diameter and contained several firm areas which on section proved to be calcified. No other calcified nodes were found. The rest of the nodes of the abdomen were examined and found to be hyperplastic but showed no areas suggestive of tuberculosis.

Microscopic Observations.—In the lungs many of the alveoli were distended with red blood cells. Others contained polymorphonuclear leukocytes and phagocytes laden with pigment and fibrin. The alveolar walls occasionally appeared to be hyalinized. Many of the bronchi were filled with red blood cells and fibrin. A mediastinal lymph node showed no abnormalities. The left upper bronchopulmonary node showed areas of caseation at the borders of which there were giant cells, epithelial cells and a thin fibrous capsule.

Marrow from the femur showed very active hematopoiesis with foci of erythroblasts and young granulocytes. There were also numerous megakaryocytes.

Final Anatomic Diagnosis.—The diagnosis was: hyperplasia of the bone marrow and of the spleen; fatty degeneration of the myocardium; severe chronic passive hyperemia of the lungs; bronchopneumonia; cloudy swelling of the kidneys; central necrosis of the liver; a primary tuberculous intestinal complex, remote, and caseous lymphadenitis of the left upper bronchopulmonary node.

No cause was found for the anemia. The case was probably one of an obsolete primary tuberculous infection of the intestine. Whether the active tuberculous disease of the mediastinal node was due to reinfection or to reactivation of older lesions subsidiary to the intestinal infection cannot be decided.

A woman, 22 years old, died of scarlet fever, and the body was examined two hours after death (G. R. Smith).

Only the observations of interest will be given. There were no palpable lymph nodes in the cervical, axillary, epitrochlear or inguinal regions.

The peritoneal surfaces were smooth and glistening. There were no adhesions or abnormal masses. The thoracic cavity contained no free fluid. The parietal pleura was gray and shining. On the dorsal and lateral surfaces of both lungs were multiple fine fibrous adhesions which were broken with ease. The base of the left lung was firmly attached to the diaphragm by dense fibrous adhesions.

The visceral pleura of the lungs was smooth, gray and glistening except in those areas to which there were adhesions. The lungs collapsed easily on exposure. The mediastinal lymph nodes were small, showed a minimal amount of anthracotic pigment and no abnormal masses. The lungs were carefully examined, both manually and by use of a fluoroscope, but no calcified areas were found either in the nodes or in the lung itself.

The mesentery was pink, smooth and glistening. Many lymph nodes were seen and could be palpated. On section they had a pinkish-yellow surface. In the angle between the terminal ileum and the cecum a large node measuring 15 by 10 by 3 mm. was found in the mesentery. On section an area of calcification measuring 2 mm. in diameter was found. Situated 6 cm. centrally from the first node, a second was found which measured 17 by 10 by 4 mm. and on section showed an area of calcification measuring 3 mm. in diameter. Careful search revealed no further abnormal lymph nodes. Fluoroscopy of the mesentery and intestines revealed only the aforementioned two calcified nodes.

The pharynx was covered with dirty brown exudate. There was visible necrosis of the tonsils. The larynx showed swelling of the mucous membranes of both the true and the false vocal cords.

The microscopic observations were of no interest in the present study.

Final Anatomic Diagnosis.—The diagnosis was: acute ulcerative and necrotizing tonsillitis; acute ulcerative pharyngitis; edema of the larynx; chronic lymphadenitis; fatty degeneration of the liver; cloudy swelling of the parenchymatous organs; periportal hepatitis; acute hyperplasia of the spleen; healed primary tuberculous infection of the lymph nodes in the mesentery, and simple cysts of the ovary.

It appears to be reasonably certain that this case was one of remote primary tuberculous infection of the intestinal tract.

REVIEW OF THE LITERATURE

Table 1 lists a group of cases selected from a larger number found in the literature. The criteria were chosen so as to insure including only such reports as satisfy the following conditions: Descriptions of isolated or selected cases have not been included nor have purely statistical studies of autopsies been done for the express purpose of studying primary infection. It was regarded as fundamental that the publication demonstrate a recognition of the modern conception of the primary infection as also of the pitfalls which meet the investigator on this subject. Thus, in his study of the frequency with which tuberculous changes are found at autopsy in the laboratory of Aschoff, Schirp [1a] reported that a routine examination produced only 48 per cent whereas careful examination of 238 subsequent cases produced 92.6

1a. Schirp, K.: Beitr. z. klin. d. Tuberk. **49**:308, 1922.

TABLE 1.—*Prevalence of Primary Tuberculous Infection of the Intestine*

Author	Year	Location	Total Number of Cases	Age Limits	Cases Primary in Lung	Cases Primary in Abdomen	Cases Primary Elsewhere	Questionable Cases	Comment
Ghon [2a]	1907-1909	Vienna	184	<15 years	162	3	2	2	Routine autopsies; 15 double primary infections including pulmonary infection
Albrecht, H.: Wien. klin. Wchnschr. **22**: 327, 1909	1909	Vienna	1,060	<12 years	?	7	?	4	All tuberculous
Ghon and Winternitz [2b]	1910-1923	Prague	666	Infants and children	567	11	3	1	All tuberculous; 24 multiple primary infections, at least 15 including intestinal infection
Opie [4]	1917	St. Louis	143	93 children, 50 adults	..	0	..	..	Routine autopsies
Opie [4]	1920	Base Hosp. No. 2, Rouen	66	20-30 years	..	18	..	0	Routine autopsies
Puhl, H.: Beitr. z. Klin. d. Tuberk. **52**: 116, 1922	1922	Freiburg	109	17 less than 18 yrs., rest over	107	2	0	0	Routine autopsies
Ghon and Kudlich [2c]	1923	Prague	31	Infants and children	30	0	0	0	All tuberculous; 1 double enteral and pulmonary primary infection
Ghon and Kudlich [2c]	1924	Prague	47	Infants and children	44	0	1	1	All tuberculous; 1 double enteral and pulmonary primary infection
Ghon and Kudlich [2c]	1925	Prague	41	Infants and children	38	0	2	1	All tuberculous
Kudlich, H.: Beitr. z. Klin. J. Tuberk. **75**: 575, 1930	1926-1929	Prague	136	<15 years	130	3	1	2	
Schuermann, P.: Virchows Arch. f. path. Anat. **260**: 664, 1926	1922-1926	Dresden	1,000	Children and adults	855	102	29	0	Eight combined upper respiratory and enteral primary infections; routine autopsies
Siegmund, H., in Henke and Lubarsch: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 4, pt. 3, p. 371	1929	Cologne	...	.....	90%	3-4%	..	..	Results of routine autopsies
Blacklock, J. W. S.: Tuberculous Disease in Children, Medical Research Council, Spec. Rep. Ser., no. 172, 1932	1932	Glasgow	283	<13 years	173	101	6	7	All tuberculous
Schirp [1]	1922	Freiburg	238	All ages	169	1	0	0	Routine autopsies
Iizuka, M.: Beitr. z. Klin. d. Tuberk. **80**: 79, 1932	1932	Berlin	2,500	All ages	?	101	?	?	Routine autopsies
Scott [3]	1930	Hongkong	300	All ages	209	32	?	11	Chinese dying of tuberculosis; 225 <10 yrs.; 4 double enteral and respiratory primary infections
Canti, R. G.: Quart. J. Med. **13**: 71, 1919	1919	London	16	Children	10	6	0	0	All tuberculous

per cent. Many publications have therefore been rejected. Particularly the publications before Ghon's fundamental studies were published have been found unfit for use here. In the past it has not been realized that the generalization from a primary infection in any part of the body may lead to a caseous lymphadenitis elsewhere. Therefore most of the multiple primary infections described must be regarded with considerable skepticism. The publications of Ghon and his co-workers [2] should be regarded as the standard for any investigations along these lines, and to Ghon's critical review of the subject [2c] the reader is referred.

But even this selected group of publications remains a material which is not homogeneous. It will be noted that some of the publications give the number of primary infections found in routine autopsies; in other cases, the number among autopsies on persons who died of tuberculosis. One fact, however, is apparent; namely, that the percentage of primary intestinal infections is much higher in the British Isles than in any other of the countries from which investigations have been reported. The three publications from England give 34.2 per cent primary abdominal infections whereas the average of the German investigations is 5.3 per cent and that of the Austrian 3.9 per cent. Scott [3] in Hongkong, China, found a percentage of 10.6; Opie [4] in a small series in St. Louis found no primary abdominal infections; Wollstein and Spence [5] in New York City found 4.3 per cent. It appears, therefore, that England takes an exceptional position in this respect. English workers are agreed that the high percentage of abdominal infections in their country is due to the unusually high percentage of tuberculous cattle. Thus, for instance, the study of the Scotch Board of Health shows that 20 per cent of the tests made on milk revealed the presence of tubercle bacilli.[6] Since raw milk is commonly used in England, the chances of infection are very great. On the Continent, where milk is regularly boiled before use, the percentage of infections by ingestion is correspondingly low. Although there have been in America no large series of carefully studied cases such as those published by Ghon, it is probable that the percentage of primary abdominal

2. (*a*) Ghon, A.: The Primary Lung Focus of Tuberculosis in Children, translated by D. Barty King, New York, Paul B. Hoeber, Inc., 1916. (*b*) Ghon, A., and Winternitz, F., cited from Ghon and Kudlich. (*c*) Ghon, A., and Kudlich, H., in Engel, S., and von Pirquet, C.: Handbuch der Kindertuberculose, Leipzig, Georg Thieme, 1930, vol. 1.

3. Scott, H. H.: Tuberculosis in Man and Lower Animals, Medical Research Council, Spec. Rep. Ser. 149, London, His Majesty's Stationery Office, 1930.

4. Opie, E. L.: Am. Rev. Tuberc. **4**:641, 1920.

5. Wollstein, M., and Spence, R. C.: Am. J. Dis. Child. **21**:48, 1921.

6. Tuberculous Infection of Milk, Medical Research Council, Spec. Rep. Ser., 189, London, His Majesty's Stationery Office, 1933.

infections in this country is low. This is shown not only by the small group of Opie [4] but also probably by the cases studied by Wollstein and Spence.[5]

It is probable that most primary intestinal infections are caused by the bovine variety of the tubercle bacillus. However, this organism is not confined to the ingestional route. It has been found in the sputum or lungs of 8 persons who, according to Lange,[7] were directly infected through contact with tuberculous cows. Griffith and Munro [8] have isolated the bovine organism in 49 cases of "pulmonary phthisis." Furthermore, some of the cases of skin tuberculosis are due to exogenous reinfection, and perhaps a considerable number of cervical nodes receive the infection in the same way. Nevertheless, the association of primary

TABLE 2.—*Prevalence of Mycobacterium Tuberculosis Var. Bovis*

Author	Year	Country	Cases	Percentage of Infections with Bovine Tubercle Bacillus	Comment
Lange [7]	1932	Germany	1,169	10.9	Own results; all ages
Park and Krumwiede [10]	1910	United States	435	7.3	Own results; all ages
Park and Krumwiede [10]	1912	World	1,500	10.0	Own results and results in cases from literature; all ages
Blacklock [9]	1933	West Scotland	165	27.3	Autopsies 7 weeks to 13 years; own results
Gaffky [9]	?	Germany	57	3.5	?
Rothe [9]	?	Germany	21	4.8	?
Ungermann [9]	?	Germany	39	5.1	?
de Besche [9]	?	Norway	51	7.8	?
Griffith [11]	1932	England	1,597	....	All ages; own results
Griffith [11]	1932	Scotland	1,221	....	All ages; own results
Ryan, C., and Voldrich, M.: J. Michigan M. Soc. **30**: 11, 1931	1931	Detroit, U. S. A.	25	0	Twenty-two children and 3 adults
Price [12]	1934	Canada	.....	10	Children from country districts

intestinal disease and bovine infection is a close one, as can be seen from the fact that of the 45 cultures of bovine tuberculous bacilli in the series of Blacklock [9] 41 were isolated from the abdominal organs (in 80.4 per cent of the total number of cases of abdominal disease). With these qualifications table 2 may be used as an indication of the percentage of primary intestinal (ingestion) infections. Again England and Scotland have shown much higher percentages than continental countries or the United States. Most of these infections occurred in childhood. In the collected statistics of Park and Krumwiede [10] the relation of human to bovine infections among adults was 62.6 to 1; among

7. Lange, Bruno: Brit. M. J. **2**:503, 1932.

8. Griffith, A. S., and Munro, W. T.: Lancet **1**:399, 1933.

9. Blacklock, J. W. S.: Edinburgh M. J. **39**:190, 1932.

10. Park, W. H., and Krumwiede, C.: J. M. Research **23**:205, 1910; **25**:313, 1911; **27**:109, 1912.

children from 5 to 16 years of age, 2.8 to 1, and among children below 5 years of age, 3.8 to 1. Likewise Griffith [11] has shown that in Scotland 33.2 per cent of the autopsies on children below 14 years of age revealed bovine bacilli whereas in persons above 15 years the percentage was 17.6.

CONCLUSION

One may therefore conclude that outside of England and Scotland infection with the bovine variety of the tubercle bacillus and primary intestinal infection are unusual except when the infected person has obtained a dairy product from an uncontrolled source. Most such infections in the United States and Canada occur in sections of the country where the milk is not pasteurized or boiled and the cows are afflicted with tuberculosis.[12] In Great Britain, on the other hand, other conditions prevail. Wilson [13] in 1932 stated that 5.2 per cent of all deaths caused by tuberculosis in England and Wales were due to the bovine organism. Jordan [14] estimated the annual loss of human life to be 2,000, and two thirds of those who have died were below 15 years of age.

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11. Griffith, A. S.: Edinburgh M. J. **39**:177, 1932.
12. Price, R. M.: Canad. Pub. Health J. **25**:13, 1934.
13. Wilson, G. S., cited from Jordan.[14]
14. Jordan, L.: Medical Research Council, Spec. Rep. Ser. 184, London, His Majesty's Stationery Office, 1933.

Case Reports

MULTIPLE OSTEOGENIC SARCOMA

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Cases in which the condition is termed multiple osteogenic sarcoma are occasionally reported in the literature. Among these are instances of Ewing's sarcoma. In others, such as the case presented by Globig,[1] the growth simulates multiple myeloma and takes origin from the marrow tissue. Both types of tumor have been classified by Geschickter [2] as nonosseous, since the process of osteogenesis is not repeated in either. On the other hand, multiple benign osteogenic tumors, such as the exostoses and chondromas, occur frequently. The occasional transition of such growths to sarcoma has been noted. Instances of bone sarcoma that is multiple in origin and depicts the process of osteogenesis have been reported only in elderly persons with Paget's disease. The occurrence in a young adult of multiple osteogenic sarcoma which terminated fatally eleven weeks after the appearance of the first symptom is therefore unusual and justifies this report.

REPORT OF A CASE

History.—A 27 year old garage helper of Spanish-Mexican parentage was admitted to the Mount Sinai Hospital on Aug. 19, 1933. He was apparently perfectly well until four weeks before admission, when he noted pain across the back, which became progressively more severe. This pain was particularly distressing on bending or straining and often radiated to both lower extremities. Two weeks after the onset of the pain he began to experience numbness of the toes of the right foot. This sensation gradually ascended until it involved the entire right leg. A few days later numbness occurred also in the left leg. Increasing weakness confined the patient to bed. Stiffness of the neck was noted, and on the day of admission there was difficulty in starting urination.

Physical Examination.—The patient was well developed and well nourished. There was a small, firm, tender mass about 1 cm. in diameter fixed to the left fourth rib. Neurologic examination revealed the following findings: a slight horizontal nystagamus that was particularly pronounced on gazing to the left, slight muscular weakness in the lower part of the face, weakness of the muscles of the trunk, paresis of both upper extremities, flaccid paralysis of the lower extremities, a positive Babinski sign on the right side and an equivocal one on the left side and a relaxed sphincter ani muscle. There were definite analgesia, hypesthesia and athermia below the level of the fourth dorsal dermatome. Position sense and vibratory sense in the lower extremities were lost. There were some stiffness of the neck and a bilateral Kernig sign.

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From the Laboratories of the Mount Sinai Hospital.

1. Globig, H.: Jahrb. f. Kinderh. **125**:90, 1929.

2. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, New York, American Journal of Cancer, 1931.

Lumbar puncture revealed xanthochromic spinal fluid and a complete block. The Wassermann tests of the spinal fluid and blood were negative. The blood count showed: hemoglobin, 90 per cent; white cells, 8,500; polymorphonuclears, 73 per cent; lymphocytes, 26 per cent, and monocytes, 1 per cent. The calcium content of the blood was 8.7 mg. per hundred cubic centimeters.

Course.—On August 23, five weeks after the onset of the pain, roentgenologic examination of the cervicodorsal region of the spine showed no definite abnormality. Laminectomy from the seventh cervical to the second dorsal vertebra failed to reveal any obstruction. The cord was swollen. Subsequently the mass on the left fourth rib grew larger, a tender tumor, 2 cm. in diameter, appeared over the left fifth rib and the tuberosities of the left humerus became tender. Following this fresh growths developed over the sternum, the left third and fourth ribs, the right zygoma and the left temporal region, producing prominence of the left eyeball. A nodule in the wall of the chest was aspirated and was reported to be a fibrosarcoma. Bence-Jones protein was not present in the urine. On September 19 a roentgenogram of the skull showed no definite abnormality. Another roentgenogram of the spine revealed no abnormality. Four doses of roentgen therapy were given over the tumors during the last week of life. The masses continued to increase in size, and weakness progressed until death occurred on October 11, eleven weeks after the first complaint.

Gross Postmortem Examination.—Skull: The calvarium varied in thickness and density, revealing irregularity of the cortex and infrequent islands of red marrow. In many places the inner plate, the diploe and the outer plate could not be delineated from one another, all of these constituents being replaced by firm yellow tissue in which there was a fine gray meshwork. Tumor masses, continuous with and projecting from the inner and outer tables, were present in the parietal, temporal and orbital regions. These were white and hard but could be cut without difficulty.

Sternum (fig. 2*A*): Numerous small, firm nodules studded the surface. Projecting into the anterior mediastinum was a large (4 cm.) tumor mass. Laterally it was continuous with masses about the clavicles and ribs, and superiorly it fused with the muscles and structures at the root of the neck. Section through the sternum revealed the loss of differentiation between cortical and spongy bone, with replacement of the red marrow by yellow tissue similar to that in the calvarium.

Ribs: Projecting from the ribs on all surfaces were tumor masses, varying from 1 to 5 cm. in diameter. Section through the bone showed a marked reduction in the diameter of the marrow canal, which was almost entirely replaced by tissue similar to that of the skull and sternum.

Left Scapula: Tumor nodules and a replaced marrow could be seen.

Vertebrae (fig. 1*B*): Along the anterior and lateral surfaces of the vertebral column were numerous large irregular fungating masses, the size of a plum, some friable and hemorrhagic, others firm and white. These were attached to the bodies of the vertebrae and infiltrated the paravertebral muscles. Frequently they fused and formed virtual sheaths about the spinal column. Section through the bodies revealed loss of differentiation between compact and spongy bone, because of replacement with dense yellow and gray tissue.

Sacrum (fig. 2*B*): The sacrum was ensheathed by a firm, irregular white tumor mass which formed a boxlike enclosure at the lower end of the spinal column. One large fungating mass was eroded, necrotic and hemorrhagic. This tumor invaded the surrounding muscles and perirectal tissues, compressing the

rectum posteriorly. Section through the sacrum also showed infiltration and destruction similar to that found in the vertebrae. There was collapse of one vertebral body.

Pelvic Bones: In both iliac fossae tumor masses arose from the iliac bones. Because of permission for only a limited autopsy the long bones were not examined. In the region of the left shoulder an irregular mass surrounding the head of the humerus was palpated.

Central Nervous System: The orbital surface of the left frontal lobe was adherent to and compressed by the underlying tumor. The spinal cord was compressed at the level of the third dorsal vertebra and between the first and fifth lumbar vertebra.

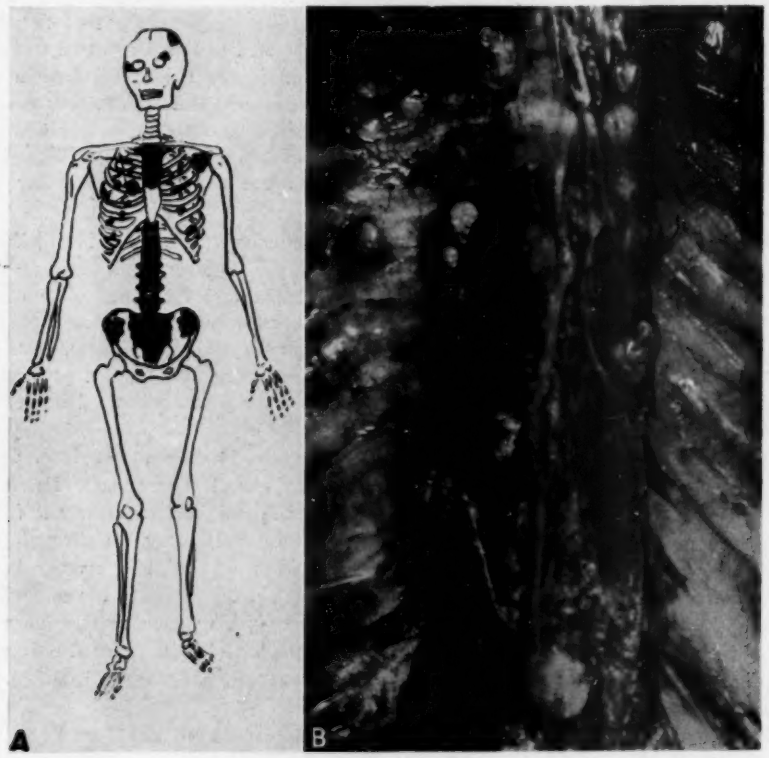

Fig. 1.—*A* shows the location of the sarcomatous growths, represented by the blackened areas. The long bones were not examined. *B* shows pedunculated tumors of the thoracic vertebrae and ribs.

Other Organs: Scattered over the surface of both lungs were a number of flat gray nodules of cartilaginous consistency, from 1 to 5 mm. in diameter, embedded in the visceral pleura. On the pericardial surface of the right auricle there was an isolated small, firm, gray tumor plaque, 1 cm. in diameter. Attached to the serosal surface of the ascending colon was a similar nodule.

Histologic Postmortem Examination.—Sections taken through grossly unchanged bone of the skull revealed a thick cortex of compact bone, regular trabeculae

and a marrow which was both fatty and cellular. Sections through bone which grossly showed a replaced marrow but which did not possess any tumor mass projecting from the cortex showed a narrow band of periosteum covering a straight surface of mature cortical bone. The diploe possessed lamellated trabeculae enclosing widened marrow spaces. Normal marrow contents were replaced by fibrous tissue, which consisted of adult fibroblasts, in many places very cellular and in others laid down in a loose, myxomatous fashion. Between these fibroblasts some sections showed a fibrillar eosinophilic matrix. The fibrils stained pink with Van Gieson's stain, indicating their connective tissue character. In places the matrix was more compact, forming osteoid tissue with slight calcification (fig. 3). These osteoid spicules were continuous with the mature bone along

Fig. 2.—*A*, tumors on the posterior aspect of the sternum. *B*, destruction of the sacral vertebrae and replacement of the marrow by neoplasm.

the endosteal border. Sections through parts of the skull where the cortex possessed an irregular, firm surface revealed mature bone growing from the cortex and lifting the periosteum. Examination of cortical tumor masses showed an elevated periosteum, displaced by underlying cellular neoplasm. The tumor cells were bizarre in shape and large, with abundant cytoplasm and polymorphic nuclei (fig. 4). There was an eosinophilic matrix, in some places fibrillar and in others homogeneous and more compact. This matrix presented osteoid metaplasia where it approximated the cortex. The bone marrow beneath the tumor sites was replaced by myxomatous, precartilaginous and osteoid fibroblastic tissue. The mature bone trabeculae possessed fine calcium lines, laid down in parallel, regular fashion.

The ribs, sternum, scapula, vertebrae and sacrum presented a similar picture; distended marrow spaces were filled by calcifying osteoid tissue, the latter forming a meshwork which enclosed a very cellular fibroblastic tissue, exhibiting pronounced atypism. The pleomorphism of the cells enclosed by the bony spicules was a prominent feature. The fibrils between the more mature fibroblasts stained pink with the Van Gieson stain. In some areas the "filling-in" tissue was myxomatous and precartilaginous. The tumor tissue growing away from the cortex was cellular, the cells being atypical and bizarre and invaded neighboring muscle and connective tissue. Examination from the outer margin of the tumors inward revealed elongated cells which became rounded, forming a loose, myxomatous structure, depositing a homogeneous matrix between cells which simulated precartilaginous tissue. Nearer the cortex the matrix was compact and osteoid, the

Fig. 3.—Section through the diploe of the skull showing the replacement of the marrow by mature fibroblastic and osteoid tissue.

spicules arising from cortical bone. The mature trabeculae of the spongy bone were narrow, eroded and irregular and possessed fine calcium lines. One section of rib showed a distinct mosaic arrangement of the lines produced by the calcium deposition.

The pulmonary nodules varied in structure from loose fibrous tissue in whorl-like arrangement to myxomatous and early cartilaginous tissue with some attempt at calcification. These lay within the visceral pleura and invaded also the pulmonary parenchyma. The nodule present in the right auricle consisted of loose fibrosarcomatous tissue with a fibrillar, eosinophilic matrix and slight calcification. The metastasis to the colon was encapsulated and limited to the serosa and possessed

a fibrosarcomatous structure, with myxomatous, precartilaginous and calcified areas. The fibrils within these metastases stained pink with the Van Gieson stain.

Other Organs: The other organs were essentially normal except for hematopoiesis and iron deposits in the spleen. The parathyroid and pituitary glands were not examined.

Postmortem Roentgenography.—Roentgenograms of the long bones, thorax, spine and skull were taken. The head of the left humerus showed an increase in density; the contour of the bone was normal. The ribs and sternum showed no changes; the cortical margins, for the most part, were distinct. The tumor masses about the bone could be outlined, and they cast the type of shadow that is produced by a purely extra-osseous fibrosarcoma. However, along the cortical attachment of

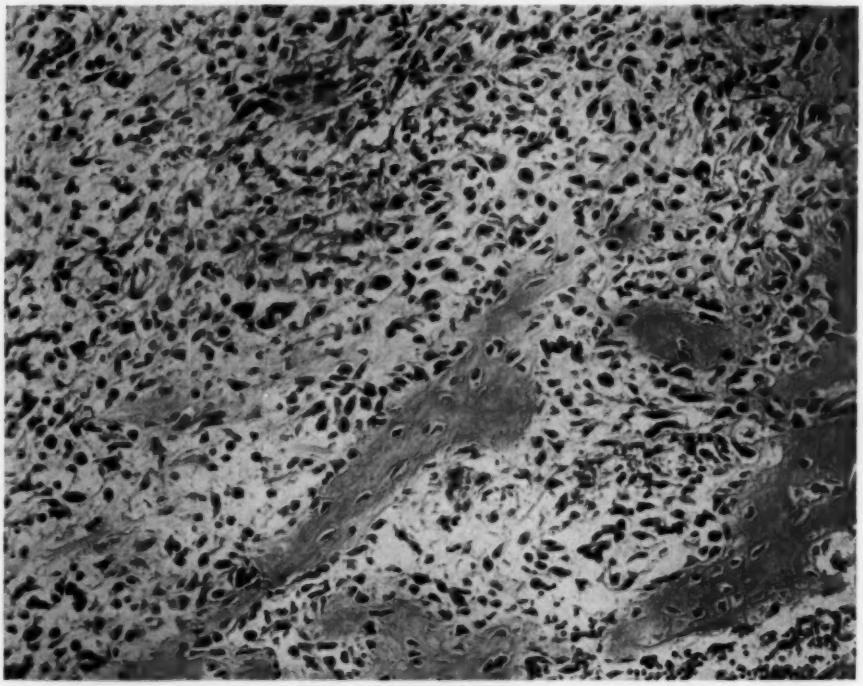

Fig. 4.—Representative microscopic appearance of the tumor masses, showing the osteogenic sarcomatous character.

some of the tumor masses fine bony spicules could be seen projecting at right angles from the cortex of the ribs. The vertebral bodies possessed irregular areas of increased density; in contour and size they appeared normal. The skull showed no changes. In the light of the postmortem observations the earlier roentgenograms were reexamined. Though areas suggesting increased density could be discerned within the vertebrae, the roentgenologist was forced to conclude that no definite abnormality could be seen.

Summary.—In a young adult, whose first complaint was pain in the back, soon followed by motor and sensory disturbances of the lower limbs, rapidly growing tumor masses in the trunk, the skull and probably

the left humerus (fig. 1*A*) developed within the short course of eleven weeks. Roentgenograms taken during this period revealed no abnormality. Postmortem roentgenographic examination of the removed bones showed some increase in density within the vertebrae and left humerus and ossification along the cortical margin of the thoracic tumors. At necropsy it was seen that the bone marrow was almost universally replaced by compact yellow and gray tissue. In many places the differentiation between cortical and spongy bone was lost. Osteogenic sarcomas ensheathed the vertebral column and grew away from the ribs, sternum, scapula and skull. Histologic examination verified the osteogenic sarcomatous nature of the tumors and revealed extensive replacement of the marrow by osteogenic tissue, frequently sarcomatous. Metastases to the lungs, heart and colon were present.

COMMENT

Tumors, such as the sarcomatous growths in this case, which arise from preosseous elements and possess a loose, myxomatous connective tissue structure with zones of immature cartilage and bone may justly be called osteogenic.

The occurrence of multiple osteogenic sarcomas is the unusual feature. Ordinarily, multiple malignant tumors are metastatic. Occasional reference is made in the literature to the presence of isolated bone metastases from a primary osteogenic sarcoma. The Registry of Bone Sarcoma, in a review of the cases on record, concluded that such instances were infrequent.[3] Widespread metastases to bone are exceedingly rare; Nunokawa's [4] case, quoted by both Kaufmann [5] and Willis,[6] cannot be accepted without reservation. In the case presented in this report the growths occurred almost simultaneously. Grossly, all were similar in appearance; microscopically, all presented analogous stages of osteogenic development. Because of the clinical and anatomic features and the fact that osteogenic sarcomas do not metastasize extensively to bone, a multicentric origin may be postulated.

If the origin of osteogenic sarcoma is to be ascribed to primitive preosseous rests, as maintained by Geschickter,[2] it would be necessary to explain this case on the basis of an enormous number of embryonal rests. This parallels Sternberg's [7] conception of the origin of the systemic diseases of the blood-forming organs, such as leukemia and lymphosarcoma. He attributed these to multiple scattered hematopoietic foci which, present during embryonal development, remain potentially active during postnatal life and under the proper stimulus proliferate. More recently, however, opinions have been advanced (literature referred to by Klemperer [8]) that the cytoplasmic reticulum present throughout the

3. Dresser, R., and Dumas, C.: Am. J. Roentgenol. **23**:65, 1930.

4. Nunokawa: Berl. klin. Wchnschr. **45**:949, 1908.

5. Kaufmann, E.: Pathology, Philadelphia, P. Blakiston's Son & Co., 1929.

6. Willis, Rupert A.: The Spread of Tumours in the Human Body, London, J. & A. Churchill, Ltd., 1934.

7. Sternberg, C.: Wien. klin. Wchnschr. **38**:40, 1925.

8. Klemperer, P., in contribution to the Medical Sciences in Honor of Dr. Emanuel Libman by His Pupils, Friends and Colleagues, New York, International Press, 1932, vol. 2, p. 655.

body (in the spleen, lymph nodes, liver, bone marrow and perivascular tissues) retains throughout adult life the multipotency of embryonal mesenchyme. Under varied stimuli cells belonging to this reticulum may undergo progressive differentiation and give rise to the systemic diseases of the hematopoietic tissues, the hemoblastoses (Orth). Similarly, in the case just described the simultaneous extensive involvement limited to one type of tissue, i. e., bone, points to a systemic blastomatous process. Thus the sarcoma, instead of arising from multiple embryonal rests, may be originated from the preosseous tissue of the periosteum, which represents a multipotent embryonal mesenchyme even in adult life.

The precise factor responsible for this imbalanced proliferation of the periosteum remains unknown. Multiple osteogenic sarcoma has been reported arising in Paget's disease (Wanke [9]). In the case just described, the replacement of the bone marrow points to the presence of a dystrophic process of the skeleton; the thinning with destruction of the bone trabeculae, their concomitant rapid reconstruction with irregular calcium deposition, the widening of the marrow spaces with replacement by fibrous tissue and osteoid metaplasia and the presence of periostitis are features which somewhat resemble, though they are not identical with, those of osteitis deformans. The marrow replacement and the development of the multiple tumors must have occurred almost simultaneously; examination of numerous sections of the skull revealed the former to be the more extensive. Whereas an underlying osteitis may have stimulated sarcoma formation, it must be borne in mind that the changes in the marrow may be secondary to the tumor growths.

SUMMARY

A case of multiple osteogenic sarcoma, with an extensive osteodystrophic process within the bone, is presented. The multiple origin of the tumor is discussed. The changes within the bone and the sarcoma formation are comparable but not identical with those seen in cases of Paget's disease.

9. Wanke, R.: Deutsche Ztschr. f. Chir. **237**:198, 1932.

Laboratory Methods and Technical Notes

HISTOLOGIC DEMONSTRATION OF UREMIA BY PRECIPITATION OF XANTHYDROL UREA IN TISSUE

ALBERT F. BROWN, M.D., AND ARAM A. KRAJIAN, LOS ANGELES

Uremia, like other metabolic disorders, is comparatively easy to detect clinically but hard to demonstrate anatomically at necropsy. Since an unsuspected azotemia is one of the most frequent causes of mistaken clinical diagnosis, the pathologist often finds urgent need for some method of proving or disproving the presence of uremia after the patient's death.

The estimation of preformed creatinine in blood obtained post mortem serves this purpose satisfactorily in most cases. However, under certain conditions, chemical tests are not feasible, for example, in embalmed bodies, in bodies in which the blood is so badly decomposed that a clear protein-free filtrate cannot be prepared or in the bodies of small infants from which sufficient blood is not obtainable. In such cases occurring in the pathologic service of the Los Angeles County Hospital, the use of a modification of the xanthydrol method described by Oestreicher in 1925 [1] has been found both convenient and effective.

In brief, the principle of the method is the immersion of the tissue in a solution of xanthydrol,[2] which is a specific and extremely sensitive reagent for the detection of urea. Crystalline xanthydrol urea is precipitated in situ, and its amount is estimated by microscopic examination of a histologic section. Xanthydrol itself is precipitated by contact with the water of the tissue, but this excess reagent is dissolved out by the alcohol used in the preparation of the section.

The test may be performed on tissue from any source, as explained in detail in Oestreicher's original work. To facilitate comparison between different cases, this study has been confined to the use of one tissue (the brain).

The original technic included fixation in alcohol and embedding in paraffin. We have substituted fixation in formaldehyde and frozen section. This reduces the time involved from a minimum of two and one-half days to a minimum of seven hours. The results are quite as satisfactory, if not more so.

From the Departments of Pathology of the Los Angeles County Hospital and of the College of Medical Evangelists.

1. Oestreicher, Alfred: Virchows Arch. f. path. Anat. **257**:612, 1925.

2. The xanthydrol was obtained from the Eastman Kodak Company.

MODIFICATION OF XANTHYDROL TECHNIC (KRAJIAN)

1. Cut thin blocks of tissue, from 2 to 3 mm. in thickness. Immerse from five to six hours in a freshly prepared solution of xanthydrol (5 Gm. of xanthydrol in 100 cc. of glacial acetic acid).[3]

2. Wash under running water for one hour.

3. Fix in a dilute solution of formaldehyde U. S. P. (1:10) six hours or longer. (In urgent cases a rapid fixation with heat may be used.)

4. Wash in tap water.

5. Cut frozen sections from 5 to 10 microns thick.

6. Transfer to glass slide.

7. Pour several drops of dehydrated alcohol over the section from a drop bottle, and blot. Repeat this process once more.

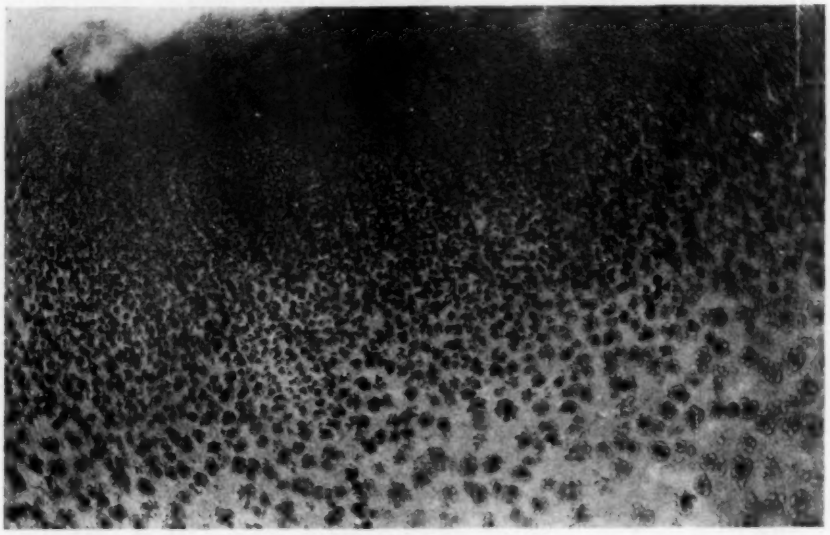

Fig. 1.—Extremely heavy xanthydrol urea precipitate in brain tissue, showing typical gradation of crystal size. Uremia was unsuspected clinically. Acute tubular necrosis occurred as the result of bismuth poisoning. Autopsy was performed two days after death. Magnification, × 70.

8. Cover with thin pyroxylin (celloidin), dipping the slide in a wide mouthed bottle containing pyroxylin.

9. Fix to the slide by blowing breath over the section.

10. Stain in a 1 per cent aqueous solution of eosin for several minutes.

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3. Recently we have developed a further slight modification of the method which is more rapid and produces better crystals. The change consists in the use of hot xanthydrol solution (70 C.) for two hours, instead of the cold reagent for from five to six hours. The period of washing in running water is reduced to five minutes, which is satisfactory for rapid work, and the fixation in solution of formaldehyde is accomplished in fifteen minutes at 70 C. The total time of the procedure need not exceed two and one-half hours.

11. Wash in tap water.
12. Dehydrate in three changes of dehydrated alcohol (drop bottle method).
13. Place in carbolxylene for two minutes.
14. Clear in two changes of pure xylene, one minute in each.
15. Mount in dammar.

Owing to the high diffusibility of urea, the tissue must be kept from contact with any fluid previous to its immersion in xanthydrol. Especially one should avoid contamination with urine during the autopsy. The blocks must be very thin, since the rate of penetration by the xanthydrol-acetic acid solution is quite slow. The use of hematoxylin to stain the nuclei has a tendency to disturb the crystals; accordingly it is preferable merely to stain the background with eosin.

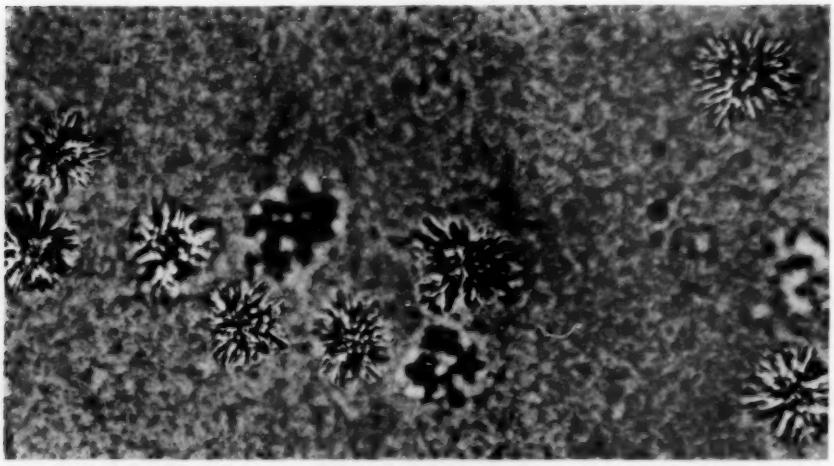

Fig. 2.—Moderately heavy xanthydrol reaction in the brain tissue of a stillborn infant with complete renal aplasia. The needles are shown in clusters. Autopsy was performed two days after death (by Dr. Hugh Edmondson). Magnification, × 950.

INTERPRETATION OF RESULTS

The appearance of the microscopic preparation in a case of well marked uremia offers a striking demonstration of the immense accumulations of tissue urea in this condition. The superficial zones, where the xanthydrol first penetrates and where precipitation is rapid, are almost completely filled with closely packed crystals of xanthydrol urea. These take the form of yellow-green needles, arranged radially in clusters. Single crystals are rarely seen. The crystal aggregates are quite tiny, but extremely numerous, at the edge of the section and, conversely, much larger but less abundant toward the depth of the tissue. Since this variation is an effect of the lessened concentration of xanthydrol at a distance from the surface, the appearance of this gradation depends on the manner of cutting the section.

Specimens from bodies of persons who did not have uremia present an entirely different picture. Careful search may reveal an occasional

group of crystals near a blood vessel or in the ganglion cells of the olivary nuclei in the medulla, but anything more than this indicates some degree of retention of urea.

Obviously, the point at which the intermediate grades of retention become actual uremia cannot be specified, except to say that a large amount of crystalline precipitate is found only in cases of true uremia.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Carl Sternberg, pathologic anatomist in Vienna and known for his work on lymphogranuloma (Hodgkin's disease), has died at the age of 64.

Fritz Reuter of the University of Gratz has been placed in charge of the Vienna Institute of Legal Medicine.

In the school of medicine of the University of Texas, Galveston, Garrett E. Williams has been appointed adjunct professor of pathology, succeeding J. M. Horn.

In the University of Oregon Medical School, Portland, Warren C. Hunter has been promoted to associate professor of pathology and A. J. McLean to assistant professor of neuropatholgy.

Lawrence W. Smith, formerly associate professor of pathology in Cornell University Medical College, has been appointed professor of pathology in Temple University, Philadelphia, to succeed the late John I. Fanz.

Society News.—The American College of Physicians will meet in Detroit on March 2 to 6, 1936.

The Second International Congress for Microbiology will meet in London on July 25 to Aug. 1, 1936. The general secretary is Dr. R. St. John-Brooks, Lister Institute, Chelsea Bridge Road, London, S. W.

At its last annual meeting the Society of American Bacteriologists elected Thomas M. Rivers president, James M. Sherman vice president and Ira L. Baldwin secretary.

The Third International Congress of Comparative Pathology will be held in Athens from April 15 to 18, 1936. In connection with the congress a twelve day cruise will be organized, beginning on April 11 at Venice, allowing attendance at the sessions of the congress and thereafter continuing until April 23, when Venice will be reached again. The places to be visited include Mount Athos, Nauplia, Olympia, Delphi, Mycenae, Corinth, Tiryns and Sparta.

The Essex County Anatomical and Pathological Society, Newark, N. J., has established an annual lectureship in honor of Harrison S. Martland, medical examiner of Essex County, and associate professor of forensic medicine in New York University.

Ella Sachs Plotz Foundation.—During the twelfth year of this foundation (1935) twenty-five grants were made, twelve of these outside of the United States. Investigations bearing more or less directly on medicine and surgery are favored. Grants may be used for apparatus and supplies needed for special investigations and for unusual expenses, including technical assistance, but not for apparatus or materials ordinarily part of laboratory equipment. Stipends for support of investigators are granted only exceptionally. As a rule, the maximum granted would be less than $500. Applications for 1936-1937 should be sent before May 1, 1936, to Dr. J. C. Aub, 695 Huntington Avenue, Boston.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Structural Changes in the Pituitary of Thyroidectomized Rats. I. T. Zeckwer and others, Am. J. M. Sc. **190**:145, 1935.

When thyroidectomy is performed in young rats it results in a stunting of body growth, an increased weight of the pituitary due both to increased solids and increased fluid content, a marked reduction or nearly complete disappearance of acidophils, an increase in the number of basic-staining cells and the appearance of great numbers of large cells filled with hyaline substance. These "thyroidectomy cells" appear, according to the special staining technic used, to be transformed cells containing blue granules. The "thyroidectomy cells" appear to be secreting and storing a secretory product which is hyaline in appearance. It is suggested that the stunting of body growth in the cretin rat may be due to loss of acidophils of the pituitary which in turn depends on loss of the thyroid secretion. Acidophils seem to disappear by degranulation rather than by frank degeneration. An abundance of thyrotropic hormone was found to be present in cretin pituitaries depleted of acidophils, an observation which rules out the acidophil cell as the producer of thyrotropic hormone. Since there is no atrophy of the adrenals in cretin rats, it is reasonable to consider that the acidophils cannot be the producers of the adrenotropic hormone. When thyroidectomy is incomplete, such changes are slight or absent.

From the Authors' Summary.

The Reactions of the Lung to Particulate Sericite. W. S. Lemon and G. M. Higgins, Am. Rev. Tuberc. **32**:243, 1935.

Sericite free from uncombined silica was given intratracheally to rabbits. The majority of the particles were less than 5 microns in diameter. A single large dose in salt solution was given. The injections did not produce pulmonary lesions similar to those found in either experimental or human silicosis. The lesions were similar to those described by Badham and Simson from the long and continuous inhalation of harmful dusts other than silica. The fibrosis was remarkably like that produced by nonsiliceous dusts in the human lung.

H. J. Corper.

Biologic Effects of Thymus Extract. L. G. Rowntree and others, Arch. Int. Med. **56**:1, 1935.

Thymus extract (Hanson) has accelerated the rate of growth and development and hastened the onset of adolescence in the offspring of treated rats and increased the fertility of parent rats. This acceleration may be noted in later litters of the second generation but is more marked in each succeeding generation under treatment. The treatment of succeeding generations of parent rats with injections of thymus extract has resulted in the amplification of the effects of thymus extract and has possibly thrown some light on the rôle of the thymus gland.

From the Authors' Conclusions.

Respiratory Metabolism of Excised Brain Tissue. S. B. Wortis, Arch. Neurol. & Psychiat. **33**:1022, 1935.

Wortis used the method of Barcroft and Warburg in studying the respiratory metabolism of nerve tissue (central and peripheral). By measuring the oxygen consumption and carbon dioxide production in small amounts of living tissue he found that the brains and minced spinal cord tissue of warm-blooded animals

normally oxidize carbohydrate and lactic acid yielding a respiratory quotient of 1. When adult brain tissue is deprived of dextrose, the rate of respiration decreases. It increases on addition of dextrose. Morphine, butyl-bromallyl barbituric acid and sodium amytal do not seem to affect the respiratory quotient or the consumption of oxygen over a period of two hours. Ethyl alcohol or injection of insulin also markedly depresses the respiratory quotient (to 0.75) as well as the consumption of oxygen, while cocaine, phenobarbital and bromides depress the consumption of oxygen by brain tissue without affecting the respiratory quotient.

GEORGE B. HASSIN.

INTRACRANIAL ARTERIOVENOUS FISTULA: DIAGNOSIS BY DISCOVERY OF ARTERIAL BLOOD IN JUGULAR VEINS. B. T. HORTON, L. H. ZIEGLER and A. W. ADSON, Arch. Neurol. & Psychiat. **33**:1292, 1935.

Intracranial arteriovenous fistula, also known by the names of angioma cavernosum, angioma arteriale racemosum, arteriovenous angioma and by many other names, is similar in structure and clinical signs to arteriovenous fistula elsewhere in the body. When suspected, it can be diagnosed without special studies of the oxygen of the blood, as the blood passing directly from the artery into the vein without the intermediation of the capillary bed contains more oxygen, and hence the venous blood on the side of the fistula appears brighter than normal. The pulsation of the internal jugular vein on the side of the lesion may be as vigorous as that of the common carotid artery, and the oxygen saturation of the blood drawn from the internal jugular vein is greatly increased.

GEORGE B. HASSIN.

SEQUENCES OF EXPERIMENTAL BACTERIAL INFARCTION OF THE FEMUR IN RABBITS. G. H. KISTLER, Surg., Gynec. & Obst. **60**:913, 1935.

Either living or dead masses of bacteria may act as emboli in producing septic or aseptic infarcts in bone. The subsequent changes are modified by infection when the infarcts are produced by infected emboli. Owing to the relatively poor collateral subchondral circulation and to the presence of open end-capillaries immediately beneath ossifying columns of cartilage, the infarcts are situated beneath ossifying cartilage, especially in the metaphyses. The diameter of the epiphyseal cartilage plate and the rate of growth within the metaphysis apparently explain the more frequent occurrence of foci in some metaphyses than in others. The size of the infarct and, if infected, the mass and virulence of the bacteria determine the extent of bone destruction and distortion. Exudative arthritis may follow the spread of infection along the epiphyseal line to the periosteum. Centers of metaphyseal growth and the adjacent portion of the epiphyseal cartilages derive their blood supply from the periosteum and nutrient vessels of the shaft. The stimulus of necrotic cortical bone and an adequate periosteal blood supply are necessary for the formation of an involucrum. Clumps of living or dead Staphylococcus aureus may produce lines of arrested growth in metaphyseal infarcts.

FROM THE AUTHOR'S SUMMARY. (WARREN C. HUNTER.)

TRANSPLANTATION OF PROSTATIC TISSUE INTO THE ANTERIOR CHAMBER OF RABBITS. N. J. HECKEL and H. L. KRETSCHMER, Surg., Gynec. & Obst. **61**:1, 1935.

Following an acute foreign body reaction, blood vessels could be seen in and about the transplants. After from six to eight weeks small grossly visible cystic spaces lined by epithelium appeared. Eventually the iris and the cornea became covered by epithelial outgrowths from the transplants, and these outgrowths showed a tendency to reproduce the original prostatic structure. Rabbits receiving injections of a soluble extract of anterior pituitary showed no additional changes. In rabbits given anterior pituitary hormone, the transplants grew more vigorously and presented hyperplastic features.

WARREN C. HUNTER.

THE EXPERIMENTAL PRODUCTION OF ENDOMETRIAL HYPERPLASIA. S. ZUCKERMAN and A. H. MORSE, Surg., Gynec. & Obst. **61**:15, 1935.

Following injections of estrin the endometrium of a castrated adolescent chimpanzee and that of a normal mangabey presented an excessive hyperplasia, identical in structural characters with the so-called Swiss cheese hyperplasia in the human female.

FROM AUTHORS' SUMMARY. (WARREN C. HUNTER.)

HYPERTENSION ASSOCIATED WITH EXPERIMENTAL OXALATE NEPHRITIS. W. M. ARNOTT and R. J. KELLAR, Brit. J. Exper. Path. **16**:265, 1935.

Repeated intravenous injection of sodium oxalate into rabbits produced hypertension, as shown by estimations of blood pressure made by the carotid loop method of van Leersum. The degree of hypertension is greater in animals fed on a dry diet of oats and bran than in those fed on a diet of cabbage.

FROM THE AUTHORS' SUMMARY.

THE SUPRARENAL IN CHOLESTEROL-FED RABBITS. W. W. KAY and R. WHITEHEAD, J. Path. & Bact. **41**:293, 1935.

Changes in the morphology and cholesterol content of the adrenals of six rabbits of each sex receiving solid cholesterol mixed with their ordinary diet have been investigated, each rabbit having a litter mate of the same sex as a control. The adrenals of the cholesterol-fed rabbits showed a gross increase in weight, volume and cholesterol content. The increased cholesterol content was established histochemically by the Schultz cholesterol reaction and by chemical analysis of whole glands. The changes in cholesterol content were qualitatively the same histologically and chemically, although the chemical variations were wider. The increase in cholesterol content of the glands was on the average one quarter of their increase in weight. The total and ester cholesterol values of the glands from the female controls were more than twice the corresponding values for the male controls. The absolute amounts of total and ester cholesterol in the glands from cholesterol-fed males were greater than the corresponding values for the cholesterol-fed females. The increase in the cholesterol content of the glands from the cholesterol-fed males, as compared with their controls, greatly exceeded the corresponding increase for the females.

FROM THE AUTHORS' SUMMARY.

MITOCHONDRIAL CHANGES IN AUTOPLASTIC LIVER TRANSPLANTS. E. S. DUTHIE, J. Path. & Bact. **41**:311, 1935.

A study of the mitochondrial changes in liver transplants shows that the surviving cells may be identified at all stages by the possession of normal mitochondrial forms. Cell death is preceded by enspherulation and enlargement of the mitochondria, with later vacuolation and disappearance; the mitochondrial changes precede those which are usually regarded histologically as characteristic of degeneration. The change from normal to spherular mitochondria is in certain cases preceded by a stage during which the cell possesses a strong affinity for mitochondrial stains. Surviving liver cells soon after transplantation show a tendency to store fat in large quantities. During this process the mitochondria become short rods which lie in close relation to the fat droplets.

FROM THE AUTHOR'S SUMMARY.

THE ACTION OF RETRORSINE ON RAT'S LIVER. J. DAVIDSON, J. Path. & Bact. **40**: 285, 1935.

The alkaloid retrorsine (found in Senecio retrorsus and certain other species) and also a liquid extract from a species of Senecio produce a series of profound changes in the liver of the rat. First there is a notable proliferation of endo-

thelial cells in the branches of the hepatic vein with consequent rupture and hemorrhage beginning in the centers of the lobules. Similar proliferative changes, though of lesser degree, may also be present in the pulmonary and cerebral vessels. As a result of the vascular lesion in the liver the hepatic cells of the central and middle zones of the lobules become necrotic. Finally, there is replacement fibrosis with regeneration of the liver cells and bile ducts, provided the animal survives for a sufficient length of time, the ultimate picture being that of an interstitial hepatitis or cirrhosis. The primary effect of the alkaloid appears to be vascular.

FROM THE AUTHOR'S CONCLUSIONS.

Pathologic Anatomy

ERYTHROBLASTOSIS IN THE NEW-BORN. H. S. ANDREWS and A. J. MILLER, Am. J. Dis. Child. **50**:673, 1935.

Four cases of erythroblastosis in which two of the patients recovered and one came to autopsy are reported. Two of the cases occurred in Negroes. These are the first cases of the disease in Negroes to be reported. In a review of 1,958 births the incidence was 1 in 490. Three of the patients had a normal period of gestation and one was premature. Two were first born. All were girls. One patient had a familial history of jaundice, and all the patients were jaundiced during the course of the disease. Hemorrhages appeared in two. The spleen was enlarged in all the patients, and the liver was enlarged in two. There was anemia in all, but no red blood cell count was below 2,000,000. There were many nucleated red cells and a tendency toward leukocytosis, the latter condition disappearing in the patients who recovered. Cells of the myeloid series varied from 1 to 8 per cent, and the color index was near 1 in all cases. Postmortem examination revealed many hematopoietic centers in the liver, kidneys, spleen and pancreas. Hemorrhages were numerous throughout the body, especially in the lungs. In the kidneys, lungs and skin were large atypical epithelial cells, an explanation of which is lacking. The bone marrow was moderately hyperactive.

AUTHORS' SUMMARY.

ANGINA PECTORIS AND HEART BLOCK AS SYMPTOMS OF CALCAREOUS AORTIC STENOSIS. E. P. BOAS, Am. J. M. Sc. **190**:376, 1935.

The syndrome of angina pectoris is common in patients with aortic stenosis. It appears to be caused, not by disease of the coronary arteries, but by narrowing of the aortic valvular opening, which impairs the blood supply to both coronary arteries simultaneously. Sudden heart failure in these patients is often followed by symptoms simulating coronary thrombosis. Auriculoventricular conduction disturbances are also often associated with the calcific form of aortic stenosis. These are determined by extension of the calcific process into the annulus fibrosus or the ventricular septum. At times a secondary infection of the diseased valve causes heart block by direct extension into the auriculoventricular bundle. Isolated aortic stenosis is probably of rheumatic origin.

AUTHOR'S SUMMARY.

ERYTHREMIA. L. F. BISHOP SR. and L. F. BISHOP JR., Ann. Int. Med. **8**:1602, 1935.

A white man, aged 65, who had been employed as an engineer in the tropics for a long time presented himself because of substernal distress. He revealed cyanosis, splenomegaly and polycythemia. The red blood cell count on most occasions was about 6,000,000, but rose as high as 9,050,000. The leukocyte count varied from 10,000 to 30,700. The hemoglobin varied from 81 to 131 per cent. Amebic dysentery developed, and he died in uremic coma. At autopsy there were found anasarca, hypertrophy of the spleen (740 Gm.), hyperplasia of the bone and marrow and arteriolonephritis. There was no extramedullary formation of bone. The enlarged heart was ascribed to the nephrosclerosis with hypertension.

There was no terminal thrombosis or hemorrhage. The increased rate of the basal metabolism was ascribed to active regeneration of the blood. It is suggested that the arteriosclerosis of the small blood vessels in the marrow caused an anoxemia which may have been etiologic.

FRANK R. MENNE.

THE OCCURRENCE OF CORONARY AIR EMBOLISM IN ARTIFICIAL PNEUMOTHORAX. T. M. DURANT, Ann. Int. Med. **8**:1625, 1935.

An unmarried woman 24 years old who showed clinical evidence of tuberculosis with cavity formation in the upper lobe of the left lung experienced convulsions for thirty seconds and became cyanotic with slowing of the pulse and respirations following a fourth unsuccessful attempt to induce artificial pneumothorax. Electrocardiographic tracings indicated infarction of the anterior wall of the left ventricle of the heart due to coronary air embolism, supporting the contentions of Rukstinat and LeCount that involvement of the coronary arteries is an important factor in air embolism complicating artificial pneumothorax.

FRANK R. MENNE.

CALCIFIED INTRADURAL CHOLESTEATOMA OF UNUSUAL SIZE. G. HORRAX, M. YORSHIS and G. R. LAVINE, Arch. Neurol. & Psychiat. **33**:1058, 1935.

In a woman, aged 43, with a defective heredity who suffered from attacks of manic-depressive psychosis, a brain tumor was removed at operation which weighed a little more than 400 Gm. and lay in the left frontoparietal region, between the arachnoid, to which it was attached, and the indented left hemisphere. It was encased in a thickly calcified shell, measured 15 by 11 by 8 cm. and proved to be a cholesteatoma. The interior was filled with brownish-yellow, amorphous, greasy material which contained glistening flicks of cholesterol. The patient's mental condition showed steady and sustained improvement after the operation.

GEORGE B. HASSIN.

RUPTURE OF A PAPILLARY MUSCLE IN THE HEART AS A CAUSE OF SUDDEN DEATH. R. R. STEVENSON and W. J. TURNER, Bull. Johns Hopkins Hosp. **57**:235, 1935.

Nineteen cases of rupture of a papillary muscle in the heart have been reported in the literature. These cases have been summarized, and one more has been described. The usual cause is coronary occlusion and infarction of the myocardium. Rupture of the left anterior papillary muscle is usually preceded by occlusion of either the anterior descending branch of the left coronary artery or the first descending branch of the circumflex portion of the left coronary. Rupture of the left posterior papillary muscle is associated with occlusion of both the right coronary artery and the circumflex branch of the left coronary artery. Sudden death occurred in half of the cases which have been reported. The condition might be suggested clinically by the sudden onset of a loud whistling to-and-fro murmur occurring in association with the signs and symptoms of coronary occlusion and cardiac infarction.

FROM THE AUTHORS' SUMMARY.

LIVER CHANGES IN EXOPHTHALMIC GOITER. G. R. CAMERON and W. A. E. KARUNARATNE, J. Path. & Bact. **41**:267, 1935.

The liver is frequently damaged in exophthalmic goiter. The cases fall into two groups according to whether or not passive venous congestion is responsible. Noncongestive cases show (1) fatty change, sometimes amounting to acute necrosis, (2) atrophy, local or general, (3) cirrhosis or (4) any combination of these. It seems reasonable to regard these changes as representing reactions to varying grades of injury the result of the thyroid derangement.

FROM THE AUTHORS' SUMMARY.

Pathologic Chemistry and Physics

CLINICAL ESTIMATION AND SIGNIFICANCE OF CALCIUM-ION CONCENTRATIONS IN THE BLOOD. F. C. McLEAN and A. BAIRD HASTINGS, Am. J. M. Sc. **189**:601, 1935.

1. The total calcium of the serum or plasma is nearly all accounted for as calcium ions and calcium bound to protein. Of these two forms the ionized calcium is of primary physiologic and clinical importance.

2. The concentration of calcium ions in the plasma is normally maintained within a relatively narrow range by a process of physiologic regulation in which the parathyroid glands play a prominent part. This range has been found, as a rule, to be from 4.25 to 5.25 mg. per hundred cubic centimeters.

3. The concentration of calcium ions in the plasma at any time is the resultant of the establishment of an equilibrium between the total calcium and total protein in the plasma.

4. A method for clinical estimation of the concentration of calcium ions by calculation from the concentrations of total protein and total calcium is presented. The results obtained by this method have been found to be in substantial agreement with the results obtained by direct observation.

5. Fluctuations in the concentration of total calcium occurring in response to fluctuations in the concentration of total protein in the plasma are necessary for the maintenance of normal concentrations of calcium ions and consequently are not in themselves of clinical importance.

6. An increase in the concentration of calcium ions in the plasma is presumptive evidence of hyperfunction of the parathyroid glands.

7. A decrease in the concentration of calcium ions in the plasma may occur as the result of hypofunction of the parathyroid glands or may be brought about by the hyperphosphatemia of uremia.

8. In all other conditions investigated, including various disorders of calcification, the concentration of calcium ions in the serum or plasma has been found to be within normal limits.

FROM THE AUTHORS' SUMMARY.

THE COAGULATION DEFECT IN HEMOPHILIA AND IN JAUNDICE. A. J. QUICKE, M. STANLEY-BROWN and F. W. BANCROFT, Am. J. M. Sc. **190**:501, 1935.

On the basis that the rate of clotting is proportional to the concentration of thrombin and that this agent is formed by the interaction of prothrombin, thromboplastin and calcium, a new method for estimating prothrombin has been developed. By employing oxalated plasma, recalcifying with an optimal amount of calcium and adding an excess of thromboplastin, prothrombin becomes the only variable in the reaction, and the amount of thrombin formed depends on the concentration of prothrombin. The clotting time, therefore, becomes a direct measure of the prothrombin. By means of this new test it has been found that the concentration of prothrombin in the blood of normal persons is remarkably constant but much lower than that in the blood of dogs and rabbits. In hemophilia the prothrombin was found to be normal in quantity; that is, the clotting time of the plasma was the same as that of normal plasma, when excess thromboplastin was added. In the plasma of patients with various types of jaundice clotting is delayed in the presence of excess thromboplastin, suggesting that the hemorrhagic tendency in jaundice is caused by a diminution of prothrombin.

FROM THE AUTHORS' SUMMARY.

VOLUME OF BLOOD IN NORMAL SUBJECTS AND IN PATIENTS WITH SCHIZOPHRENIA. J. M. LOONEY and H. FREEMAN, Arch. Neurol. & Psychiat. **34**:956, 1935.

It has been assumed that decreased oxygenation or failure of the tissues to utilize oxygen properly may be the cause of schizophrenia. Either the cells

cannot obtain enough oxygen because of changes in the oxygen-combining power of the hemoglobin, or the total volume of blood in the body may be decreased. Looney and Freeman studied the concentration of hemoglobin and the total volume of blood. The volume of the blood was studied during a period of seven months, at intervals of three months, in eighty-seven men with schizophrenia and in twenty-nine normal men. The hemoglobin content for the patients was practically the same as that for the controls, and so was the total volume of blood, the volume of plasma and that of red cells; the body weight remained practically constant. When the blood was measured on the basis of surface area, the authors found "a decrease in the total circulating volume of whole blood in patients with schizophrenia considered as a group."

GEORGE B. HASSIN.

Immunology

SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS. K. LANDSTEINER and J. JACOBS, J. Exper. Med. **61**:643, 1935.

Experiments on sensitization of guinea-pigs with simple chemical compounds are described. Positive effects were obtained by the administration of small quantities, namely, fractions of milligrams, of 1:2:4 chloro-di-nitrobenzene, p-nitroso-dimethylaniline, 1:4:4 tri-nitrobenzene, picryl chloride, four di-chloro-di-nitrobenzenes and a number of other aromatic compounds. Several substances chemically similar to those enumerated gave negative results. The first-named compound is known to produce hypersensitiveness in human beings, a large number of cases having been observed in factory workers. The mechanism of these effects is discussed.

FROM THE AUTHORS' SUMMARY.

IMMUNE BODIES FOR ROUGH AND SMOOTH TUBERCLE BACILLI. R. TUNNICLIFF, K. M. HOWELL and N. HIRSCHBERG, J. Infect. Dis. **56**:111, 1935.

These experiments suggest that the opsonic test, when the S strain of tubercle bacilli is used, may not always indicate tuberculosis but may indicate the grade of infection. The complement-fixation test with antigen from an S strain is specific in the diagnosis of tuberculosis but is not diagnostic of the grade of infection. Few immune bodies for the R strain were demonstrable in the serums examined.

FROM THE AUTHORS' CONCLUSION.

LATENT TUBERCULOUS IMMUNE BODIES IN NONTUBERCULOUS INDIVIDUALS. A. B. BAKER and M. WETHERBY, J. Infect. Dis. **56**:153, 1935.

The blood of 100 consecutive adult patients with chronic arthritis was tested with the complement-fixation test for tuberculosis both before and after a course of intravenous vaccination with streptococci. Fifty-one of the patients showed a positive skin test with old tuberculin and had a negative complement-fixation test before the institution of any therapy; of these, 26, or 51 per cent, had a positive complement-fixation test after the intravenous streptococcic vaccination. This indicates that specific antibodies for tubercle bacilli may be exfoliated into the circulation of patients who at some previous time have had a tuberculous infection as indicated by the positive skin test. If antibodies indicate resistance against tubercle bacilli the results suggest a persistent protection against these bacilli long after the original infection. Forty of the patients showed a negative Mantoux test and a persistently negative complement-fixation test after the intravenous streptococcic vaccination. The occasional positive complement-fixation test in apparently normal persons may be explained in some instances on the basis of a nonspecific exfoliation of antibodies which were produced during a previous tuberculous infection.

FROM THE AUTHORS' CONCLUSIONS.

THE PREPARATION OF SPECIFIC BACTERIAL CARBOHYDRATE SUBSTANCES BY ELECTROLYSIS. C. H. YEN and T. J. KUROTCHKIN, J. Infect. Dis. **56**:238, 1935.

Saline suspensions of Staphylococcus albus, Monilia tropicalis and Klebsiella pneumoniae become clarified after being subjected to the passage of a strong direct electric current. The clarification is attributable to the action of the sodium hypochlorite formed during electrolysis.

The specific carbohydrates from M. tropicalis and K. pneumoniae have been prepared in the following way by electrolysis: The clarified cell suspensions after electrolysis are first centrifugated, then filtered through a Seitz asbestos filter and finally dialyzed in running water for forty-eight hours. The dialyzed clear solutions are protein-free; apparently the bacterial proteins are thrown out of the solutions at anode during electrolysis. When 4 volumes of 95 per cent alcohol are added to 1 volume of the acidified clear dialysates protein-free precipitates of a whitish color are obtained. The precipitates are found to be water-soluble polysaccharides, nonantigenic and capable of reacting with specific antiserum to a high titer.

By varying the duration of the electrolysis it was found that with both K. pneumoniae and M. tropicalis the maximum yield of the specific carbohydrates is obtained after electrolysis for forty hours.

The specific carbohydrates from these two micro-organisms have also been prepared by the well known alkaline extraction method. The preparation of the specific carbohydrates from K. pneumoniae by electrolysis is more efficient than that by the alkaline extraction method, but with M. tropicalis the reverse holds true.

The serologic titer of the carbohydrate from K. pneumoniae is not much affected by prolonged electrolysis in sodium chloride solution; the serologic activity of the substance obtained from M. tropicalis is readily destroyed during electrolysis in sodium chloride solution. When sodium nitrate is employed instead of sodium chloride in electrolysis the serologic titer of the carbohydrate from M. tropicalis is not much affected by prolonged electrolysis.

The liberation of the specific bacterial carbohydrates during electrolysis in sodium chloride solution is attributable to three main factors: (1) the sodium hypochlorite formed during electrolysis which causes lysis of the bacterial and yeast cells; (2) the sodium hydroxide accumulated during electrolysis, which combines with the bacterial protein; (3) the attraction of the alkali proteinate to the anode and its precipitation from the solution, leaving the specific bacterial carbohydrate in the solution. In the case of electrolysis in sodium nitrate solution probably only the last two factors are involved, as no significant amount of sodium hypochlorite is formed.

FROM THE AUTHORS' SUMMARY.

THE CARBOHYDRATES OF THE ROUGH AND SMOOTH FORMS OF STAPHYLOCOCCUS AUREUS. R. E. HOFFSTADT and W. M. CLARK, J. Infect. Dis. **56**:250, 1935.

A type-specific carbohydrate was isolated from the undissociated yellow form of Staphylococcus aureus. It was not in itself antigenic but gave type-specific precipitates with its specific antiserums. When adsorbed on collodion and injected into animals it produced a serum which agglutinated the S type of the organism but not the R type. From the R type of the organism a carbohydrate was isolated which was not antigenic in itself and which did not react with any serums used in the experiment.

The carbohydrates differed not only antigenically but in the manner in which they separated from the proteins of the respective organisms. It was necessary to use repeated precipitations to free the R carbohydrate from the protein fractions. It was almost impossible to free them from a persistent brown color which appeared in the residues. It is not improbable that the color was due to a fraction of the pigment which was changed during dissociation, leaving the R type colorless, and that it became oxidized during the extraction. The end-product of the two extractions differed chemically; the S carbohydrate was more soluble in hot

water than the R carbohydrate; the S contained less nitrogen than the R and was precipitated with barium hydroxide while the R was not.

It was shown by one of us (R. E. Hoffstadt) that the R form of the organism differs in pigment formation from the parent form. On dissociation it lost the virulence of the S type and gained the power to ferment lactose and levulose, which the parent type did not have. Tillett and Francis isolated from the R form of Pneumococcus a fraction which they called the C fraction. It did not produce precipitins but was precipitated with serums of types I, II and III. They decided it was common to all types of pneumococcus. In this it differs from the type isolated from the R dissociant of this study. Julianelle and Weighard called attention to the fact that the substances isolated by them did not precipitate in all serums.

During dissociation radical changes take place in the virulence and biochemical reactions of Staph. aureus. It does not seem impossible that a chemical change might take place which would render the carbohydrate inert. We have shown that grossly the two carbohydrates differ in the manner in which they separate from the proteins, in their solubility in water and in their reaction to barium hydroxide. More complete analysis, which is now in progress in our laboratory, may show further differences.

These facts show that the undissociated S type of Staph. aureus contains a soluble specific carbohydrate. The white R dissociant of that form contains a carbohydrate which differs from that of the S form chemically and which is apparently inert antigenically.

FROM THE AUTHORS' SUMMARY.

ANTIGENIC REACTIONS OF STAPHYLOCOCCUS AUREUS AND ITS VARIANTS. R. E. HOFFSTADT and G. P. YOUMANS, J. Infect. Dis. **56**:288, 1935.

The rough and gonidial forms of Staphylococcus aureus afford no protection against infection with the smooth virulent form of the organism.

Each strain of this organism contains heat-stable and heat-labile antigenic fractions. The rough and the smooth strains have a heat-stable antigenic fraction in common. Gonidial forms contain a heat-labile fraction in common with the rough and smooth forms.

FROM THE AUTHORS' CONCLUSIONS.

THE RELATION OF ALLERGY TO THE ANTIBODY CONTENT IN ANIMALS VACCINATED WITH B C G. B. J. CLAWSON and A. B. BAKER, J. Infect. Dis. **56**:297, 1935.

In animals vaccinated with B C G there was no proportionate or necessary relation between bacterial allergy as manifested by the intracutaneous tuberculin reaction and antibodies in the blood as indicated by agglutination and complement fixation.

FROM THE AUTHORS' SUMMARY.

THE RELATION OF THE INCIDENCE OF SERUM DISEASE TO THE SQUARE ROOT OF THE AMOUNT OF ANTISERUM INJECTED. T. D. GERLOUGH, J. Infect. Dis. **56**: 317, 1935.

Analysis of the exhaustive reports of Hunt and of Weaver shows that the incidence of serum disease produced with antidiphtheritic serum is closely correlated with the square root of the amount injected. The relationship may be expressed approximately by the simple equation $I = K\sqrt{C}$ in which I is the incidence, C the amount injected and K the constant for a particular serum.

FROM THE AUTHOR'S SUMMARY.

METEOROLOGICALLY CONDITIONED VARIABILITY OF SEROLOGIC TESTS IN SYPHILIS. E. T. HOVERSON, W. F. PETERSEN and DE L. SACKETT, J. Lab. & Clin. Med. **20**:337, 1935.

A single negative Wassermann or Kahn test of the blood means little so far as ruling out syphilis is concerned. Some persons having syphilis exhibit rather

striking variations in the serologic tests, and these variations take place in terms of days. In those cases in which variations occur there seems to be no absolute agreement between the Wassermann and the Kahn tests. It is believed that the variations are conditioned by meteorological changes.

FROM THE AUTHORS' CONCLUSIONS.

PREVENTION OF INTRANASALLY INOCULATED POLIOMYELITIS OF MONKEYS BY INSTILLATION OF ALUM INTO THE NOSTRILS. C. ARMSTRONG and W. T. HARRISON, Pub. Health Rep. **50**:725, 1935.

The instillation of 4 per cent sodium aluminum sulfate into the nostrils of monkeys resulted in the survival of seventeen of twenty-three animals, while only three of nineteen nonprepared controls survived similar intranasal inoculation with the virus of poliomyelitis. Poliomyelitis tended to develop later and to run a slower course in the alum-prepared group than in the nonprepared controls. The protective action of the alum solution is believed to be due to an alteration which decreases the permeability of the mucous membrane of the nose rather than to an antiseptic action.

FROM THE AUTHORS' SUMMARY.

THE MECHANISM OF IMMUNITY TO FILTERABLE VIRUSES. A. B. SABIN, Brit. J. Exper. Path. **16**:70 and 84, 1935.

Mixtures of the virus of vaccinia and an excess of immune serum incubated for periods of one, seven and fourteen days and then centrifugated at 14,000 revolutions per minute for three hours yielded sediments in which the virus was present in practically undiminished concentration and fully infective. The presence of complement in the mixture did not affect the outcome. Similar results were obtained with the virus of pseudorabies and B virus. The protective substance was present uncombined in undiminished concentration in suitable filtrates of mixtures of antivirus serum and an excess of virus which had been incubated for various periods of time. The evidence obtained in these experiments does not support the conception that the virus combines with the protective substance in vitro and is thereby inactivated. It appears rather that under the conditions of these experiments there is no union between the virus and the protective substance in antivirus serum.

The data presented in this communication are not in agreement with the conception that the protective substance in antivirus serum combines with the virus—at least not in a manner which is similar to known antigen-antibody reactions. Immunity to the virus is intimately bound up with the cell. Both the protective substance and the virus are fixed by the tissue; if the fixation of the virus precedes that of the protective substance infection of the cell is not prevented; if the fixation of the protective substance precedes, or perhaps even when it is simultaneous with, that of the virus infection is prevented. The mechanism of the antagonism is obscure.

FROM THE AUTHOR'S SUMMARIES.

THE RELATIONSHIP BETWEEN THE COMPLEMENT AND THE OPSONIN OF NORMAL SERUM. J. GORDON and F. C. THOMPSON, Brit. J. Exper. Path. **16**:101, 1935.

Experiments have been described showing that five different treatments of normal guinea-pig serum all affect opsonin and complement differently, and that in each case the opsonin is the more resistant.

STAPHYLOCOCCUS TOXINS AND ANTITOXINS. A. T. GLENNY and M. F. STEVENS, J. Path. & Bact. **40**:201, 1935.

Certain strains of Staphylococcus produce two toxins for which there are corresponding antibodies.

FROM THE AUTHORS' CONCLUSION.

THE EFFECT OF ANTISTREPTOLYSIN ON INFECTION OF MICE BY HAEMOLYTIC STREPTOCOCCI. E. W. TODD, J. Path. & Bact. **40**:243, 1935.

High titered antistreptolysin serums protect mice to a limited extent against infection by homologous or heterologous strains of the hemolytic streptococcus. The protection is group-specific but not type-specific. The protection is not due to antitoxin, and it is probably not due to antistreptolysin. The nature of the protective antibody is unknown.

FROM THE AUTHOR'S SUMMARY.

STREPTOCOCCUS TOXIN-ANTITOXIN FLOCCULATION. R. A. Q. O'MEARA, J. Path. & Bact. **40**:371, 1935.

The flocculation is not associated with neutralization of the erythrogenic toxin by its antitoxin, nor is it a precipitation of bacterial protein in the accepted sense of the term. The flocculating substance is a specific streptococcic product per se which plays a part in streptococcic infection and to which antibodies are developed in the course of human disease. Many characteristics of the flocculating substance are those of a toxin, but direct evidence of its toxicity is lacking.

FROM THE AUTHOR'S SUMMARY.

THE RELATIONSHIP OF THE EOSINOPHILE LEUCOCYTE TO ALLERGY AND ANAPHYLAXIS. A. C. P. CAMPBELL, A. M. DRENNAN and T. RETTIE, J. Path. & Bact. **40**:537, 1935.

Local and general eosinophilia are frequent, though not constant, accompaniments of allergic inflammation in guinea-pigs. Local eosinophilia is not produced by an injection of foreign protein if an allergic reaction does not occur; and blood eosinophilia is produced on reinjection only under conditions compatible with the occurrence of an allergic reaction, i. e., an interval of time following the first injection which corresponds to that usually found necessary for the development of sensitization. Preexisting blood eosinophilia appears to have no protective action against the occurrence of allergy or general anaphylaxis.

FROM THE AUTHORS' CONCLUSIONS.

Tumors

TUMOR OF THE NEUROMYO-ARTERIAL GLOMUS. V. RAISMAN and L. MAYER, Arch. Surg. **30**:911, 1935.

Three cases of glomal tumor, the first to be recorded in the American literature, are added to the list of twenty-six cases previously reported. This benign tumor, first authentically described by Masson in 1924, develops from a structure normally present in the skin and known as the glomus. It is probably identical with the "painful subcutaneous nodule" reported by Wood in 1812, referred to by Paget in 1853 and described by other authors as "colloid sarcoma" (Heller), "painful subcutaneous fibroma" (Tillaux), "angiosarcoma" (Kolaczek), "painful subcutaneous angioma" (Monod) and "subungual perithelioma" (Müller). Thirty-three instances have been collected by us and reported as "probable but unproved cases of glomal tumor." The glomal tumor has a characteristic microscopic appearance and a typical clinical course. It is small, usually not more than ½ inch (1.27 cm.) in diameter, of slow growth and long duration. It may be situated in any part of the body, but the favorite site is beneath the nail. There it gives rise to pain of varying intensity and radiation. In the severe cases the pain is agonizing, almost paroxysmal, and radiates upward to the shoulder, even to the heart. When the tumor is subungual, the pain is usually much less severe. Changes of temperature, particularly cold, increase the severity of the pain. Invariably, the tumor is sensitive to pressure. In some cases it is associated with neurologic or vascular changes such as hyperesthesia and increase in the temperature of the skin. In two cases there was slight atrophy of the entire arm, and in one, Horner's syndrome.

Examination reveals a pea-sized tender tumor, which, when subungual, has almost always a peculiar bluish tinge showing through the translucent nail The x-ray picture of the growth in this situation may show a minute crater-like depression of the dorsal cortex of the terminal phalanx. Glomal tumor is easily differentiated from most of the cutaneous neoplasms. Only two of the latter may cause difficulty: first, the unusual single small neuroma, superficially located; second, the subungual melanoblastoma. This very malignant tumor at one phase of its development is characterized by a bluish-black spot beneath the nail which might be mistaken for a glomus. Operation and microscopic section will at once make the differential diagnosis possible.

FROM THE AUTHORS' SUMMARY.

TRANSMISSION OF MYELOID LEUKEMIA OF MICE: ITS RELATION TO MYELOMA. J. FURTH, J. Exper. Med. **61**:423, 1935.

A transmissible strain of myeloid leukemia of mice is described. It can be readily passed from diseased to healthy mice by the transfer of tissues that contain live cells; but when the latter are not present inoculation fails. Inoculation is successful in almost every mouse the resistance of which is lowered by irradiation of the mouse with x-rays. It is often successful in mice related to the animal in which the spontaneous leukemia took its origin, and occasionally successful in mice unrelated to it. The systemic diffuse disease (myeloid leukemia) is produced only by intravenous inoculation of relatively large doses, whereas subcutaneous or intraperitoneal inoculation results in the formation of tumors composed of myelocytes with basophil granules [multiple myeloma], in other words malignant blood cells of the strain described. Intravenous inoculation of susceptible mice with small doses or similar inoculation of somewhat resistant mice with larger doses results in the formation of tumors composed of myelocytes (multiple myeloma).

FROM THE AUTHOR'S SUMMARY.

THE RELATION OF LEUKOSIS TO SARCOMA OF CHICKENS. E. L. STUBBS and J. FURTH, J. Exper. Med. **61**:593, 1935.

A transmissible strain of sarcoma (strain 13) is described that took its origin in a chicken inoculated with leukosis of strain 1. The virus of strain 13 produces sarcoma in the breast muscle into which it is injected, and after intravenous injection it produces diffuse sarcomatosis of the spleen, bone marrow and several other organs. The available evidence suggests that strain 13 is caused by a single virus with ability to produce diffuse endothelial growth in the blood-forming organs associated with extensive hematomas and erythroblastic proliferation. The strain can be readily transmitted by material free from live cells and preserved by drying. Amounts of blood as small as 0.00001 cc. from chickens with sarcomatosis of the internal organs transmit strain 13 by the intravenous route. By implantation sarcoma of strain 13 can be transferred to chickens that are resistant to repeated inoculations with leukosis viruses.

FROM THE AUTHORS' SUMMARY.

ON THE FREQUENCY OF OCCURRENCE OF OCCULT CARCINOMA OF THE PROSTATE. A. R. RICH, J. Urol. **33**:215, 1935.

In 292 consecutive autopsies on men 50 or more years of age dying from a wide variety of causes in the Medical, Surgical and Urological Services of the Johns Hopkins Hospital during the past three years routine microscopic sections disclosed frank carcinoma of the prostate in 41 cases (14 per cent). There is little doubt that a thorough search throughout each gland would have brought to light an even greater number of cases of this tumor, since in many cases it was so small that it was not seen macroscopically at the time of autopsy. The number of cases discovered, however, indicates plainly enough that cancer of the prostate is considerably more frequent than is ordinarily supposed. In 65.8 per cent of the 41 cases the tumor was not recognized clinically, having been in most cases of a

size too small to have produced symptoms or to have attracted attention on physical examination. The tumor was most often found near the outer margin of the gland, and even when only a few millimeters in size, showed a tendency to invade the capsule.

FROM THE AUTHOR'S SUMMARY.

THE MORPHOLOGY OF SMALL PROSTATIC CARCINOMA. R. A. MOORE, J. Urol. **33**:224, 1935.

Carcinoma of the prostate occurs more frequently with increasing age until 29 per cent of patients in the ninth decade of life who come to autopsy show the lesion. It is intimately associated with senile atrophy and probably is derived from epithelial cells which have previously undergone atrophy. It is predominately a lesion of the posterior lobe but may arise in any portion of the gland. Carcinoma and benign enlargement of the prostate are two distinct diseases, which occur independently. Carcinoma of the prostate rarely occurs in acini already hyperplastic and involved in benign enlargement. Perineural lymphatic invasion in the capsule of the prostate is one of the earliest changes in carcinoma; invasion of the vesicles and distant lymphatic invasion are late manifestations.

FROM THE AUTHOR'S CONCLUSIONS.

THE INFLUENCE OF THE PARENTERAL ADMINISTRATION OF CERTAIN SUGARS ON THE p_H OF MALIGNANT TUMORS. C. VOEGTLIN, R. H. FITCH, H. KAHLER, J. M. JOHNSON and J. W. THOMPSON, Nat. Inst. Health Bull., no. 164, 1935, p. 1.

The technic described in the second paper of this bulletin for the estimation of the hydrogen ion concentration in tissues of living animals by means of the capillary glass electrode was applied to the following tumors: the Jensen rat sarcoma, the Flexner-Jobling rat carcinoma, the Walker rat sarcoma 325 and a spontaneous adenocarcinoma of the albino mouse. In fasting animals the parenteral administration of dextrose causes a pronounced decrease in the p_H of certain tumors, indicating that the p_H is dependent on their supply of dextrose. Experiments with the Jensen sarcoma and the Flexner-Jobling carcinoma indicate that d-fructose, d-mannose, maltose and to a lesser extent d-xylose also produce a decrease in tumor p_H, whereas l-arabinose, d-galactose, lactose and sucrose are inactive. Besides decreasing the p_H, the active sugars also increase the lactic acid content of these tumors. Attention is called to the serious errors involved in p_H estimations made on tissue extracts or macerations and in the use of the colorimetric method of those employing quinhydrone or hydrogen electrode. The authors discuss the possible significance of the experimental data with regard to (1) the occurrence of necrosis in malignant tumors and (2) the destructive action of cancerous tissue on adjoining normal tissue.

FROM THE AUTHORS' SUMMARY.

THE GROWTH OF THE JENSEN RAT SARCOMA IN SUBCUTANEOUS AND INTRAMUSCULAR TRANSPLANTS. W. R. EARLE and C. VOEGTLIN, Nat. Inst. Health Bull., no. 164, 1935, p. 47.

In histologic studies on rats each of which had been inoculated intramuscularly and subcutaneously with the Jensen sarcoma, it was found that the growths which arose from the two types of inoculation were markedly different. While the subcutaneous inoculations grew into tumors generally completely encapsulated and of the type usually associated with the Jensen sarcoma, the intramuscular inoculations produced tumors which actively invaded the muscle without interposition of any collagenous capsule. Further, of the two types of inoculation, the intramuscular gave rise to a palpable tumor at an earlier average date than did the subcutaneous inoculation and to a tumor that was markedly more vigorous in its growth. Attention is called to the fact that these intramuscular tumors, from the point of view of experimental cancer research, simulate the spontaneous clinically malignant sarcoma markedly more closely than do the encapsulated subcutaneous tumors com-

monly used. This difference in behavior after the two types of inoculations is not limited to the Jensen sarcoma but is noted in at least one other type, the Walker mammary carcinoma 256.

FROM THE AUTHORS' SUMMARY.

ATYPICAL GROWTH OF CERVICAL EPITHELIUM OF THE MONKEY FOLLOWING INJECTIONS OF OVARIAN HORMONE COMBINED WITH TRAUMA. MILTON D. OVERHOLSER and EDGAR ALLEN, Surg., Gynec. & Obst. **60**:129, 1935.

Ovarian follicular hormone stimulates epithelial growth in the female genital tract and breast. Large amounts of female sex hormones exist in the blood of both female and male patients with cancer. Experiments indicate that ovariectomy greatly decreases the incidence of spontaneous mammary cancer in mice. Hofbauer has reported that transplants and extracts of the anterior hypophysis produce in guinea-pigs proliferation of cervical epithelium. The authors studied the changes in the cervical epithelium of monkeys given repeated injections of female sex hormones at the same time as chronic trauma of the cervix was produced. In three ovariectomized animals there were marked downgrowth of squamous epithelium and overgrowth and epidermization of the cervical glands at the junction of the squamous and glandular epithelium. In the same group an animal which was not subjected to cervical trauma but which was infected displayed pronounced papillary gland overgrowth and epidermoid change with atypical cells. A monkey subjected to trauma alone showed slight epidermization; one which had been ovariectomized and traumatized but not given the hormone had a wholly normal cervix. The sections have been studied by a number of pathologists but there is no agreement as to the significance of the epithelial changes, or as to whether they resemble early cancer or not.

WARREN C. HUNTER.

PRECANCEROUS AND CARCINOID LESIONS OF THE CERVIX OF THE UTERUS. ERLE HENRIKSEN, Surg., Gynec. & Obst. **60**:635, 1935.

Henriksen made a study of the cervix in an endeavor to correlate as accurately as possible the histologic picture and the subsequent clinical course. He states that the basal layer of the normal cervical epithelium shows more mitosis and hyperchromatosis than most observers have recognized, but he looks on these as evidence of normal growth and repair. In the adult, age seems to have no influence on the thickness of the epithelial layer of the cervix. There are no constant histologic changes in the cervix during pregnancy. Inflammatory processes bring about increased cellular activity in the basal layer. Distortion of the histologic pattern follows hot douches, cauterization and local therapy. Leukoplakia of the cervix may display cellular features suggestive of cancer, although invasion is always lacking. In two cases the microscopic changes were similar to those of Bowen's disease and extramammary Paget's disease, a condition rarely, if ever, described in the cervix. The value of the biopsy is enhanced by immediate fixation, which prevents nuclear chromatolysis from the action of the alkaline secretions of the cervix, and by making serial sections, particularly in very early cancer. The importance of taking specimens of adequate size is stressed. The danger of infection, dissemination or stimulation of the tumor growth and of hemorrhage is not so great as it was formerly considered. The Schiller test is undoubtedly of value in the diagnosis of early cancer, but whether absence of carcinoma can always be determined by the test is doubtful. The descriptive term "precancerous" should be applied only to those processes which may, but do not necessarily, become malignant.

WARREN C. HUNTER.

LYMPHANGIOMA OF THE GREAT OMENTUM. ALBERT H. MONTGOMERY and IRVING J. WOLMAN, Surg., Gynec. & Obst. **60**:695, 1935.

Two cases of lymphangioma of the omentum are reported. In the literature reports of fifty-one cases were found. About two thirds of the recorded cases occurred in children. The symptoms are due either to the size of the growth or to twisting of the pedicles.

WARREN C. HUNTER.

Technical

AN IMPROVED TECHNIQUE FOR SILVER IMPREGNATION OF RETICULUM FIBERS. H. C. WILDER, Am. J. Path. **11**:817, 1935.

Sensitization of sections with uranium nitrate before impregnation with silver has reduced the time required for staining reticulum fibers to ten minutes and has eliminated the necessity of heating the ammoniacal silver. In this way the difficulty of staining sections which are inclined to wash off the slides has been greatly lessened. The substitution of phosphomolybdic acid for potassium permanganate is recommended for such sections as still persist in coming off the slides. Hydrobromic acid substituted for oxalic acid in the pretreatment, and uranium nitrate substituted for the sodium carbonate of Foot's reducing solution, have improved the staining of argyrophil fibers. The method presented here is in routine use in this laboratory. It has proved a time-saver and has given even more satisfactory results than the older methods. FROM THE AUTHOR'S SUMMARY.

MOULAGES: THE PREPARATION AND SERVICE TO THE NEUROPATHOLOGIST. JOSEPH H. GLOBUS, Arch. Neurol. & Psychiat. **34**:355, 1935.

Globus finds Economo and Poller's method of preparation of moulages useful. A new casting substance makes the casts inexpensive. In brief, the method is as follows: A brain preferably fixed in a solution of formaldehyde (this should be washed out before it is brought in contact with the molding substance) is covered first with petrolatum by means of a brush and then with plaster of paris. When the latter is hardened it is removed from the brain and its interior covered with shellac. The molding substance (negocoll, sometimes reenforced with celerit) is plastered over the specimen, allowed to harden for about fifteen minutes and then placed in the casting mixture. For this Globus recommends a mixture of white wax, rosin, plaster of paris (calcium sulfate, desiccated) and hydrated finishing lime, with which he successfully replaced the much more expensive hominit used by Economo.

GEORGE B. HASSIN.

SIMPLE METHOD OF STAINING MACROSCOPIC BRAIN SECTIONS. H. E. LEMASUIER, Arch. Neurol. & Psychiat. **34**:1065, 1935.

By treating large sections of the brain, previously hardened in a solution of formaldehyde, first with a warm solution of phenol and a 1 per cent solution of ferric chloride (for two minutes) and later, after washing in running water, with a 1 per cent solution of potassium ferrocyanide, LeMasuier obtained a beautiful differential stain of the gray and white substances. The method is also suitable for staining pathologic brains. As the slices are preserved in 70 per cent alcohol they may be used for teaching purposes and display in museums.

GEORGE B. HASSIN.

DESICCATION OF SERUM AND OTHER BIOLOGIC PRODUCTS IN THE FROZEN STATE. W. J. ELSER, R. A. THOMAS and G. I. STEFFEN, J. Immunol. **28**:433, 1935.

Experiences of many years relating to drying of biologic materials in the frozen state are related in detail. The present method, which was evolved after many trials, has proved its applicability for clinical and commercial purposes. Antigens and immune serums were kept for many years in a dry and frozen state without loss of potency. The sensitizing substance contained in the serums from asthmatic patients and those with hay fever were preserved for at least three years. Bacteria (including species as sensitive as gonococci and meningococci) and yeast have been preserved for years without changes in their biologic properties. Attempts to preserve spirochetes and protozoa have not been successful. I. DAVIDSOHN.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Oct. 14, 1935

PERCIVAL BAILEY, *President, in the Chair*

EDWIN F. HIRSCH, *Secretary*

PRESIDENTIAL ADDRESS: THE RELATIONSHIPS OF THE PATHOLOGIST TO THE CLINIC. PERCIVAL BAILEY.

I am conscious of the honor you have done a clinician in electing him to this office. Although in no sense of the word a pathologist, I have always had a serious interest in this field and have already had occasion to lament its neglect by the physician (Cushing, H., and Bailey, P.: Tumors Arising from the Blood Vessels of the Brain, Springfield, Ill., Charles C. Thomas, Publisher: Angiomatous Malformations and Hemangioblastomas, 1928).

In the not far distant past morbid anatomy was considered the indispensable door through which one had to pass to the medical clinic, just as the path to the surgical clinic led through the dissecting room. In Paris the aspiring young surgeon still must spend several years as voluntary prosector in the dissecting room, and the young internists continue for many years to examine their own patients post mortem. In our country, however, anatomy has been abandoned by the surgeon in favor of physiology, and morbid anatomy by the internist, first for bacteriology and later for chemistry—so much so that for many years American students have been going to Vienna for further training in this discipline.

While there is an element of fashion in the almost universal neglect of morbid anatomy, it is evident that the spectacular successes of bacteriology and chemistry have justified the homage paid them in the clinic. Nevertheless, they have overshadowed unduly the older pathology, which still remains the indispensable basis of diagnosis, especially in my own field of nervous diseases. The complication of knowledge by the newer developments has aided also in crowding clinicians out of the field, and has given rise to ever increasing numbers of professional pathologists, better trained and hence better able to form an accurate opinion of morbid processes.

But it is not alone the clinicians who have lost interest in morbid anatomy. The pathologists themselves tend to abandon it for pathologic physiology, immunology or chemistry, giving as an excuse that the subject is worked out and that further discoveries of importance are not to be made. That, as usual, the field has been abandoned before it has yielded its full harvest has been proved in the realm of my own interests by Lindau's recent discovery of the complex now universally known as Lindau's disease, by the important studies of Masson and his pupils on the covering cells of the nervous system, and by many others.

While I believe that many more discoveries will be made in the field of morbid anatomy which will justify its cultivation for its own sake, no one will question its importance for the clinic. If the pathologist neglects it, the clinician will again cultivate it for his own purposes. In common with the study of all branches of science, that of morbid anatomy, although a justifiable end in itself, has also practical usefulness as a handmaiden of the clinic.

You are doubtless better aware than I of these two aspects, not of morbid anatomy alone, but of pathology in a broader sense, but I should like to relate to you an experience of my own with them. In 1922, when I returned to Cushing's clinic, he proposed to me that I create a laboratory for the study of his surgical material. His dissatisfaction with the general pathologic laboratory had arisen in the following way: A specimen removed at operation would be sent to the laboratory and a report would come back with the diagnosis glioma. The same diagnosis would be made on a second specimen, but, whereas the patient from whom the first tumor was removed would be dead within six months, the second patient would be well and thriving. The diagnosis, in other words, was practically useless to the clinician, who wanted the information as a basis for prognosis. A classification was needed which could be used for this purpose and, since Cushing did not have the necessary technical training to attack the problem and had been unable to interest the pathologist in it, he suggested that I attempt it.

Let me state that the pathologist was quite correct in his diagnosis in each of the aforementioned cases. He was following the system of Mallory, who classifies tumors according to the nature of the essential neoplastic cells composing them. The culmination of Mallory's system is to be found in his classic "Principles of Pathologic Histology" (Philadelphia, W. B. Saunders Company, 1914) and it is both logical and workable, but it does not take into account the rapidity of growth of neoplastic cells, a matter of the utmost importance to the clinician who must give a prognosis. It was this element of prognostic importance which I was to try to introduce.

The results of the study of the gliomas which I pursued for many years, both while in Cushing's clinic and later, have been embodied in a monograph (Bailey, P., and Cushing, H.: A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis, Philadelphia, J. B. Lippincott Company, 1925) and in numerous separate articles in various medical journals. To my surprise they were immediately criticized by clinicians, not on the basis of oversimplification this time, but as too complicated, the clinician being usually willing to complicate his knowledge only so far as it has practical usefulness. Many pathologists (e. g., Spatz, in Munich), on the other hand, still maintain that my classification is too simplified to take into account the kaleidoscopic variations of the structure of these tumors which, they maintain, are so infinite as to make any classification impossible.

The same difficulty has arisen in regard to the tumors of the meninges. Cushing has launched the term "meningioma" for them. This simple and all-inclusive word has some justification in the fact that most tumors of the meninges are rounded, circumscribed and noninvasive. They have a certain clinical unity regardless of their histologic structure. The term cannot be used, however, to include the diffuse sarcomatous tumors of the meninges without losing all practical as well as histologic connotation. On the other hand, the histologic terminology of Mallory and Penfield, who call these tumors arachnoidal fibroblastoma, is no better in accord with the facts, since the tumors may be composed of bony or even of cartilaginous cells. Under these circumstances I have developed two vocabularies. When speaking of these tumors to a clinician I use the term "meningioma," knowing that he will understand a circumscribed, noninvasive tumor. When speaking to a pathologist, I am careful to be much more specific in my terminology. I proceed in much the same way concerning the gliomas. I may speak to a clinician of a pinealoma, but to a pathologist I should be careful to specify whether the tumor is of embryonic or adult type or even teratomatous. The clinician is not interested in these fine distinctions since they have no known clinical connotations.

Another result of the loss of interest in morbid anatomy is the neglect of technic, and this may explain in part the scarcity in this field of new discoveries,

which are so apt to follow new technical developments. A prevalent attitude was expressed by a well known pathologist one day in Mallory's laboratory. He is reported to have remarked, when looking at one of Mallory's beautiful preparations, "Oh, Frank! Show me a thick section of formalin-fixed tissue stained with hematoxylin and eosin." The opposite view is embodied in men like Mallory and also Masson, who maintains that hematoxylin and eosin should be banished from the laboratories. Masson's attitude is exaggerated. One might even find a place for the old teased preparations, although I should not go so far as Cushing and Eisenhardt and recommend such an anachronism as superior for diagnostic purposes to stained preparations. Nevertheless progress is greatly aided by technical perfection, as I know by personal experience as well as by knowledge of the history of medicine.

For example, the development of a specific impregnation method by Hortega enabled him to discover in the brain certain peculiar cells theretofore overlooked, which he called oligodendroglia. By means of this method, slightly modified by my pupil Bucy, I was able to prove that the peculiar structure of certain gliomas was due to their being composed of more or less embryonic oligodendroglia. Again, while studying cysts of the cerebellum, I was able, by use of a new impregnation method of Perdrau, to determine that many of them were hemangiomatous and not gliomatous as formerly supposed. I made this discovery independently of Lindau, although I did not, of course, see these cysts in the general relationship which he so brilliantly elucidated. Finally I might mention that the development of a sharply specific method for the eosinophilic granules in the hypophysis enabled me to confirm the constant association of an eosinophilic adenoma with the clinical syndrome of acromegaly as had been previously asserted by Benda. If Cushing's supposed pituitary basophilism is ever proved to exist it will be by the use of some such specific method for demonstrating the basophilic granulation of the hypophysis. Recently I have written for histologic preparations from the hypophysis in each case reported, and have been disgusted that such imperfect preparations could ever be supposed adequate to decide a scientific problem.

The best example probably of what perfected technic can do is to be found in the field of neuropathology. What a vast store of information has been accumulated as the result of the technical achievements of Nissl, Weigert, Marchi, Golgi, Ramón y Cajal and Hortega! The whole field of anatomy, physiology, pathology and semeiology of the nervous system has been illuminated. Recently faddism has led to the neglect of the old-fashioned neuro-anatomy and neuropathology in favor of reflex physiology and electrophysiology. It is, however, increasingly apparent that the results of these newer developments can be interpreted only when controlled by the older methods. It is by the combination and collaboration of neuro-anatomy and neuropathology with the newer technics that ever more accurate knowledge of the working of the master tissue in health and disease will be acquired.

I think I need not labor this point. The advantages of special technic in the study of the blood are also obvious. Perhaps an attempt to develop technics adequate to the study of other organs may yield equally fruitful results. As a case in point I may mention the quartz rod method of Kniseley which is transforming knowledge of the spleen. Kniseley is interested in methodology; some one else will apply his discoveries in the clinic and pathologic laboratory.

The double aspect of pathology, utilitarian and theoretical, leads to complications also in the practical working relationships in hospitals, clinics and universities. In the small outlying private hospital I imagine there is little friction. The pathologist is primarily there to be of use to the clinicians and holds his job only so long as he makes himself useful. In the university, however, the situation is somewhat different. There we have usually a group of men who are supposed to pursue the subject of pathology for its own sake. They are apt to resent,

and justly so, being at the beck and call of clinicians who want information when they need it and insist on getting it then.

But adjustments can be made, as they should be in a democratic country, and, while not perfect from a logical standpoint, they often turn out to be advantageous to both parties. They suppose only tolerance and an ability to see the other man's point of view. The arrangement at the University of Chicago seems to me very good. The surgical and clinical pathologic work is done by the surgical and medical staffs. These laboratories are equipped to deliver definite information for practical purposes in a minimum of time. They are under the charge of men who are vitally interested in that information, and if they do not function properly the clinicians have only themselves to blame. The pathologists, on the other hand, are free to pursue investigative interests regardless of the immediate emergency. Contact is maintained by conferences at which both groups are present and discuss problems of common interest. The arrangement seems to me as nearly ideal as possible. It keeps the clinician conscious of the importance of pathology as a living, growing discipline and still maintains the interest of the pathologist in the practical possibilities of his studies without burdening him with their routine application.

In this way both groups may contribute harmoniously to what must be the common purpose of all intelligent men—to make the path as easy and agreeable as possible in this vale of tears through which all must plod. My pleasure with the cooperation and good will of the pathologists in my own university is surpassed only by my admiration of the hospitality and broadmindedness of the members of this organization which not only includes many clinicians among its members but even honors them with its presidency.

You will pardon me that I have, in the course of these brief remarks, spoken so much of myself. I felt the necessity of justifying in some way my sudden elevation to this position, and I knew no other way of doing it than to show you that I have really been interested in pathology for some years, and it is on the basis of this sincere interest and in spite of my incompetence that I make bold to accept your invitation and preside over your deliberations.

THE REACTION OF ANTIBRAIN SERUM WITH SPINAL FLUIDS FROM INSANE PATIENTS: A PRELIMINARY REPORT. JULIAN H. LEWIS.

An antibrain serum which was very sensitive to brain tissue was allowed to react with 466 spinal fluids obtained from patients in psychopathic hospitals. The purpose in these experiments was to find whether evidence of disintegrative processes in the brain could be obtained by disclosing brain tissue antigen in the spinal fluid by means of an antibrain serum. Of the 466 specimens 36, or 7.7 per cent, gave positive complement-fixation reactions with the antiserum. Of the 466 specimens, 244 were from patients with dementia paralytica, 17, or 7 per cent, of which reacted positively. Of 61 specimens from patients with undiagnosed psychoses, 5 reacted; of 8 specimens from patients with mental deficiency and psychosis, 2 reacted; of 27 from patients with alcoholism and psychosis, 6 reacted. Neither of 2 specimens from patients with alcoholism without psychosis reacted. One specimen each from 27 patients with dementia praecox, 18 with cerebral syphilis and psychosis, 9 with cerebral arteriosclerosis and psychosis, 8 with manic-depressive disturbances, 8 with organic disease of the brain, 4 with epilepsy and 3 with psychoneurosis reacted. No reactions were obtained with spinal fluids from 13 patients without psychosis, 4 with paranoia or a paranoid state, 4 with drug addiction without psychosis, 3 with psychoneurosis, 3 with mental deficiency without psychosis, 3 with multiple sclerosis, 2 with postepidemic encephalitis and psychosis, 2 with senile psychosis, 2 with reactive depression, 1 with hysteria, 1 with tuberculosis and psychosis, 1 with traumatic psychosis and 1 with chorea and psychosis.

DISCUSSION

A. WEIL: Did you separate the gray and the white substance in preparing your antibrain serum?

E. A. PRIBRAM: I attempted to prepare antibrain serum in horses by injecting rabbit's brain. A definite skin reaction was produced in some animals. Also a positive ninhydrin reaction was observed. These were interesting results, but I had no positive serum reactions. What controls did you use?

J. H. LEWIS: A separation of the gray and white tissues of the brain is not necessary. An antibrain serum will react with any brain tissue. The controls used for the antibrain serum were: (1) known brain suspensions for titer and (2) spinal fluids from undiseased and nonpsychiatric adults.

A NEW METHOD FOR PREPARING MULTICOLORED CORROSION SPECIMENS OF THE KIDNEY. JOSEPH K. NARAT.

After the blood vessels and ureter of a kidney have been thoroughly flushed with water, they are filled with stained solutions of a vinyl resin in acetone and the organ immersed in water for twenty-four hours. The water solidifies the injected solutions. The kidney is placed in concentrated commercial hydrochloric acid for four days. All the tissues are digested, and only casts of the blood vessels, ureter and calices containing the injected substance remain.

This method requires little time and employs acid-fast dyes, thus offering the opportunity of preparing multicolored specimens.

The complete report will be published in the *Anatomical Record.*

CHROMAFFIN TUMOR OF THE MEDULLA OF THE ADRENAL GLAND, WITH ASSAY OF THE EPINEPHRINE. ELEANOR M. HUMPHREYS.

A 51 year old woman had a fracture of the neck of the left femur with little displacement. A few hours later symptoms developed which were thought to indicate cardiorespiratory embarrassment. Some of the findings on physical examination suggested beginning cardiac failure due to hypertensive cardiac disease, but there were other features like those of traumatic shock. The systolic blood pressure fluctuated between 130 and 268 mm. of mercury, and the diastolic pressure, between 80 and 110. There was no history of previous cardiac disorders. The peculiar mixture of symptoms persisted; the temperature rose from 97 to 109 F., and the patient died forty-eight hours after the injury. The necropsy revealed only moderate cardiac hypertrophy and minimal arteriosclerosis and nephrosclerosis. The lungs were slightly edematous, and some pulmonary vessels contained fat emboli. There were no significant changes in other viscera except the right adrenal gland. This weighed 35 Gm. and consisted of a cystic gray-brown globular tumor with islands of yellow cortical tissue in its capsule. The tumor tissue resembled adrenal medulla, and its chormaffinity was demonstrated histochemically. A chemical assay twenty hours after death demonstrated from 2.5 to 3 mg. of epinephrine per gram of fresh tumor, or from 65 to 90 mg. in the entire tumor. While it is not possible to demonstrate beyond doubt a causal relationship between the tumor and the clinical symptoms, this case is strikingly like others in which similar symptoms and death followed minor accidents or operations, and in which, excepting the presence of a chromaffin tumor, the cause of death was not accounted for. Some of the symptoms may have been due to fat embolism, but the amount of fat in the lungs was small, and no fat emboli were seen in other viscera.

DISCUSSION

A. ARKIN: Were there changes in any of the other endocrine glands, especially the pituitary?

E. M. HUMPHREYS: The pituitary seemed slightly larger, but the usual histologic preparations revealed no changes. Serial sections were not made.

Regular Monthly Meeting, Nov. 11, 1935

PERCIVAL BAILEY, *President, in the Chair*

EDWIN F. HIRSCH, *Secretary*

DUPLICATION OF THE SPINAL CORD WITH SPINA BIFIDA AND SYRINGOMYELIA. ARTHUR WEIL and WARREN B. MATTHEWS.

This article was published in full in the December issue, page 882.

MULTIPLE MYELOMA WITH TUMOR-LIKE AMYLOIDOSIS. A. H. ROSENBLUM and J. D. KIRSHBAUM.

There are thirty-nine reports of multiple myeloma associated with amyloidosis. The amyloid deposits may be local or generalized. In thirteen reports the amyloid deposits were described as tumor-like masses. Amyloidosis may be primary or idiopathic when there is no apparent underlying suppurative or neoplastic condition, and secondary or symptomatic when a preexisting cause is known to be present, such as neoplasms, tuberculosis or chronic suppurative processes. Local amyloidosis, at first regarded as idiopathic, on closer study has been associated with myeloma, a condition easily overlooked in the presence of extensive amyloid deposits in the tumor itself. In multiple myeloma the deposits of amyloid may be isolated nodules or solid masses. The case presented was that of a white woman, aged 39 years, who had a tumor mass over the manubrium sterni and clinical manifestations of renal insufficiency. She had marked azotemia and proteinuria. The blood pressure was not elevated; Bence-Jones protein was not present, and the proteose found in the urine could not be identified. The congo red test showed marked retention of the dye, and over 90 per cent disappeared from the blood stream. Tissues from the tumor mass in the sternum revealed amyloidosis with numerous foreign body giant cells. Tissues later were taken from a rib to determine the cause of the amyloidosis. The histologic diagnosis was lymphocytic myeloma with focal deposits of amyloidosis. Roentgen examination of the skull, ribs and long bones showed areas of osteolytic destruction typical of multiple myelomas. Clinically the patient had most of the features of multiple myeloma, modified by the complicating amyloidosis, with a nephrotic syndrome terminating in death from renal insufficiency.

When a patient appears to have primary or so-called idiopathic amyloidosis, either local or diffuse, repeated biopsies are advisable to exclude the possibility of an underlying myeloma. If multiple myeloma is present, the congo red test should be performed in order to detect associated amyloidosis. In multiple myeloma, instead of the typical Bence-Jones protein, atypical proteins found in the urine have the same diagnostic significance as the former.

TUMORS OF THE BREAST IN CHILDREN. CHESTER C. GUY.

Mammary neoplasms in the young are sufficiently infrequent to make noteworthy the two observed. One was a pure lipoma of the breast in a boy aged 4½ years. It had been growing for six months. True lipoma is one of the rarest mammary tumors at any age.

Malignant tumors of the breast in children have been reported, but a critical analysis of many of the reports casts doubt on the accuracy of the diagnosis. Six carcinomas of the breast in children below the age of 19 have been recorded. Sarcoma of the breast is more common, the usual type being fibrosarcoma. Round cell sarcoma has diagnostic difficulties and is undoubtedly often confused with anaplastic small cell carcinoma. A lymphosarcoma of six months' duration was observed in the breast of a feebleminded girl aged 15 years. Metastasis had already occurred in the axillary glands and the other breast. There was a history of trauma occurring three years previously. The patient died about two weeks after mammectomy. Photographs were shown of both patients before operation and of the tumors after removal.

DISCUSSION

R. H. JAFFÉ: I have in mind two young children with mammary tumors. In one the nodules were leukemic infiltrations; in the other the lesions thought to be round cell sarcoma were demonstrated by postmortem examination to be mammary metastases of a neurocytoma.

PONTILE ABSCESS: A REPORT OF TWO CASES. B. W. LICHTENSTEIN and HOWARD ZEITLIN.

Abscesses in the pons varolii, as in the medulla and spinal cord, are rare when compared with abscesses elsewhere in the central nervous system. Of forty-five abscesses of the brain found in a series of 7,349 necropsies at the Cook County Hospital from Jan. 1, 1929, to July 1, 1935, only two were in the pons. The vascular supply and the location of the pons probably explain its relative immunity. The clinical symptoms of pontile abscess are polymorphous and depend on the tracts and nuclei involved. In the majority of cases there is hemiplegia alternans inferior, known also as the Millard-Gubler syndrome. In some cases the tegmental syndrome of Foville is present and in rare instances, as in our second case, the clinical syndrome is due only to the involvement of the pyramidal tract.

In both cases the abscess had a capsule of young connective tissue and was associated with nonsuppurative encephalitis in its immediate vicinity, mild degenerative changes of the ganglion cells and marked meningitis.

The experiments of Weed and others as well as facts from human pathology (Hassin) conclusively prove that the flow of tissue fluid is from the Virchow-Robin spaces to the subarachnoid spaces of the brain and spinal cord. The transport of gitter cells and waste substances into the subarachnoid space causes a reactive phenomenon in the meninges known as aseptic meningitis.

DISCUSSION

DR. A. WEIL: Statistics on the frequency of cerebellar abscess seem to vary in reports of different origin. Some investigators have reported as many as one third of all abscesses of the brain to be cerebellar, the most frequent next to abscesses of the temporal lobe. Granted such frequent occurrence, one finds it hard to accept the theory which was advanced that the rarity of pontile abscesses is due to the vascular supply of the pons by branches of the basilar artery, the same artery being a main source of blood supply for the cerebellum also.

It is difficult to be convinced that the meningitis in the first case was aseptic meningitis. The number and type of leukocytes within the subarachnoidal spaces depend on the virulence of the invading micro-organism, on the immunity reaction of the invaded tissue, etc. The microscopic picture which was presented here may be seen in mild leptomeningitis following invasion by pneumococci or other micro-organisms. It does not correspond to the facts that an infection must always spread from the brain to the meninges following the flow of cerebral fluid along perivascular channels. The opposite way of infection is seen in meningoencephalitis, for example, in cases of lethargic encephalitis. One might well argue that a pontine abscess may arise secondarily to meningitis by invasion of a meningeal vessel and subsequent formation of a thrombus within the pons; likewise a pus-forming micro-organism of low virulence may produce purulent encephalitis by spreading along perivascular channels.

R. H. JAFFÉ: When I saw these lesions at the time the brain was removed I considered them tumors. Consequently cultures were not made.

Book Reviews

Failure of the Circulation. By Tinsley Randolph Harrison. Cloth. Price, $4.50. Pp. 396, with 60 illustrations. Baltimore: Williams & Wilkins Company, 1935.

During the last ten years Harrison and his associates at Vanderbilt University have investigated in the laboratory and ward various phases of heart disease, especially failure of the heart. From time to time their results have been published in various journals. They are now, after careful editing, arranged in book form, making a volume that is a credit to American medical scholarship. The work is really a defense of the "back-pressure" theory of congestive heart failure as opposed to the hypothesis of "forward-pressure." The latter hypothesis, it will be remembered, had a vigorous proponent in Sir James Mackenzie. Largely through his influence it came about that a goodly proportion of British and American medical men (not so many, the reviewer thinks, as Dr. Harrison believes) accepted the view of forward pressure. Dr. Harrison's proof is advanced on the basis of a restudy from a physiologic, pathologic, chemical and physical point of view of many of the old questions involved. Among the major phenomena of congestive heart failure are discussed such subjects as enlargement of the heart, fatigue in enlarged hearts, edema and dyspnea. The latter topic has been divided by the author into so many different types that it is not always easy to see the reason for his classification. There are also considered minor cardiac symptoms, such as cyanosis, altered heart tones, arrhythmias and blood pressure, as well as symptoms in organs other than the heart. There is a short discussion of failure of the coronary circulation, especial attention being devoted to angina pectoris. Among the helpful features of the book are the summaries that are found at the end of many of the chapters and the ingenious figures and diagrams. While frankly stating his position as a champion of the theory of back pressure, Harrison is a fair opponent. He outlines the arguments of the other side and freely acknowledges that they often contain much truth. He admits that there are many gaps in the present knowledge of congestive failure that can be filled only by further investigation. The book is in no sense a treatise or textbook on heart disease. Its scope is limited. The clinician, however, will find many hints as to diagnosis and treatment that are to be commended particularly because they are accompanied by an explanation of the symptom or the reason for the effect of the drug or other remedial agent. The historical introduction also deserves praise if for no other reason than that it does belated justice to one of the most accomplished of the earlier writers on diseases of the heart, James Hope, whose views as to the theory of back pressure were stated with unusual clearness as far back as 1831. Several typographic errors that have slipped by the proof-reader can easily be corrected in a later printing.

Forensic Medicine: A Text-Book for Students and a Guide for the Practitioner. By Douglas J. A. Kerr, M.D., F.R.C.P.E., D.P.H.; Lecturer on Forensic Medicine in the School of Medicine of the Royal College, Edinburgh; The Police Surgeon and Medical Referee to the City of Edinburgh. Price, $5.50. Pp. 311, with 40 plates, 4 color plates and 30 other figures. London: A. & C. Black (New York: The Macmillan Company), 1935.

This book is adapted particularly for Scotland and England. This is true especially of the first two chapters, which deal with professional ethics, the workings of the General Medical Council and details of legal procedure in case of death; it is also evident in the discussion of drunkenness in relation to traffic accidents.

The rest of the book has a more general applicability. The usual ground of English books on forensic medicine, including insanity and toxicology, is covered. Among the other topics discussed may be mentioned identification; sudden death; wounds and injuries; blood and hairs; accident, suicide and murder; gunshot wounds and identification of firearms; burning, lightning and electric shock; heat, cold, starvation and neglect; asphyxia; pregnancy and abortion, and sexual offenses. The presentation is clear and competent. Of the seventy-nine illustrations, mostly photographic, by far the greater number present realistically the gross external appearances in cases observed by the author of suicide and murder by violent means. This feature is well nigh overdone, but there are no illustrations of wounds by machine-guns! There are four good reproductions of photographs in natural colors (blood spectrum, postmortem lividity and the stomach in lysol poisoning and in hydrocyanic acid poisoning). There is no description of fatal injuries due to automobiles. In the discussion on page 57 of sudden death in relation to the circulatory system there is no mention of the importance of coronary disease. The book makes no claim to be an exhaustive treatise on forensic medicine. As indicated in the title, it is intended for the instruction of medical students and for guidance of the practitioner in medicolegal work. It will be found to be a useful book within its sphere.

A Bibliography of the Poem Syphilis Sive Morbus Gallicus by Girolamo Fracastoro of Verona. By Leona Baumgartner and John F. Fulton. Price, $5. Pp. 157, with portrait and 10 illustrations. New Haven, Conn.: Yale University Press, 1935.

This beautifully printed and meticulously edited bibliographic monograph is dedicated by the authors to Arnold C. Klebs, who contributes a biographic introduction on the famous syphilologist Fracastoro, a sixteenth century humorist and philosopher of the great Padua school of learning. He not only gave a name to the disease that had vexed and baffled physicians since ancient times, but, in his various tracts, anticipated by inspiration and intuition the present fundamentals of pathology, treatment and prophylaxis in the vast group of contagious and infectious diseases. He recognized the carriers of contagion by simple conclusion and therefore commands a lasting historical interest as one of the founders of preventive medicine.

Fracastoro's poetic lucubration on syphilis occupies a separate chapter in the history of the medical sciences. The authors of the present bibliography, recognizing this fact, have surveyed the entire field of literature bearing on the famous Italian, with the greatest exactness and with such completeness as is rare even in this day. The numerous editions of the poem in various languages are elaborately recorded, many with reproductions of titles in facsimile and with evaluating and critical annotations. In addition, the compilers have recorded more than a hundred carefully selected and abstracted references to Fracastoro's life and to the position and importance of his work in medicine.

No future student of the subject can overlook this bibliographic monograph. It has been done with consummate skill and recognizes all available and even many remote sources, indicating the location of rare editions. The bibliography may well be described as the last word on the subject. Much the same is true of the printing of the volume by the Yale University Press.

A Textbook of General Bacteriology. By Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago and in Rush Medical College. Eleventh edition. Cloth. Price, $6. Pp. 825, with 202 illustrations. Philadelphia: W. B. Saunders Company, 1935.

To keep abreast with new knowledge many changes have been made in this edition. The section on viruses and virus diseases had to be rewritten. The parts concerning immunity, streptococci, Salmonella, Brucella, Rickettsia and spirochetes have been subjected to revision and expansion. Bacterial variation is dealt with

in a new and separate chapter. It is emphasized that while variability in bacteria presents many important problems "the general stability of the types of epidemics would seem to postulate a similar stability in the types of the invading micro-organisms." It would be well if the general principle expressed in this statement should receive careful consideration in discussions of the specificity of pathogenic bacteria. Referring to his treatment of the problem of bacterial nomenclature, which he states some bacteriologists will regard as too conservative, Jordan points out that the names for bacteria used by recent authors, especially British authors, is further "evidence of the impossibility of achieving a unified nomenclature except by international agreement." Hence his hesitation in using in a textbook names that have not won a fairly general acceptance. The number of pages is six more than in the preceding edition. The remark in the note in the ARCHIVES (**14**:135, 1932) on that edition that there is no sign of "any relaxation in the concise clearness, the judicial restraint and the remarkable accuracy of the presentation" still holds true. In the face of a book like this one doubts the validity of the statement that the day of the textbook on bacteriology by the single author is past.

Books Received

LABORATORY METHODS OF THE UNITED STATES ARMY. Edited by James Stevens Simmons, B.S., M.D., Ph.D.; Major, Medical Corps, United States Army; Director of Laboratories, Army Medical Center; Director of the Department of Preventive Medicine, Army Medical School. Associate editor, Cleon J. Gentzkow, M.D., Ph.D.; Major, Medical Corps, United States Army; Chief of the Division of Chemistry, Army Medical School. Approved by the Surgeon-General of the United States Army. Fourth edition, greatly enlarged and thoroughly revised. Price, $6.50. Pp. 1091, with 70 illustrations. Philadelphia: Lea & Febiger, 1935.

ANGIOSTOMIE UND ORGANESTOFFWECHSEL. Prof. Dr. E. S. London. Pp. 193, with 54 illustrations. Moscow: Verlag des All-Union-Instituts für experimentelle Medizin, 1935.

THE PATHOLOGY OF INTERNAL DISEASES. William Boyd, M.D., M.R.C.P. (Edin.), F.R.C.P. (Lond.), Dipl. Psych., F.R.S. (Can.), Professor of Pathology in the University of Manitoba, Pathologist to the Winnipeg General Hospital. Second edition, thoroughly revised. Price, $10. Pp. 904, with 335 illustrations. Philadelphia: Lea & Febiger, 1935.

FAILURE OF THE CIRCULATION. Tinsley Randolph Harrison. Cloth. Price, $4.50. Pp. 396, with 60 illustrations. Baltimore: Williams & Wilkins Company, 1935.

BIJDRAGE TOT DE KENNIS VAN DE PRIMAIRE LONINGECTIES EN VAN DE FREQUENTIE DER TUBERCULOSE IN INDIË. Inaugural Thesis. Soegiri, Institute for Pathology, School of Medicine, Batavia, Java. Pp. 174. Batavia-C: Drukkerij Centrum, 1935.

OVER DE RESULTATEN BEREIKT MET FRIEDMAN'S MODIFICATIE VAN DE REACTIE VAN ASCHHEIM-ZONDEK. Inaugural Thesis. Machteld Agricola Pet, Institute for Pathology, School of Medicine, Batavia, Java. Pp. 131. Batavia-C: Drukkerij Centrum, 1935.

CLASSICAL CONTRIBUTIONS TO OBSTETRICS AND GYNECOLOGY. Herbert Thoms, M.D., Associate Professor of Obstetrics and Gynecology, Yale University. With a foreword by Howard A. Kelly, M.D., Professor Emeritus of Gynecology, Johns Hopkins University. Price, $4. Pp. 265, with 57 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1935.

EPIDEMICS AND CROWD-DISEASES: AN INTRODUCTION TO THE STUDY OF EPIDEMIOLOGY. Major Greenwood, D.Sc., F.R.C.P., F.R.S.; President of the Royal Statistical Society; Professor of Epidemiology and Vital Statistics in the University of London; formerly Medical Officer in the Ministry of Health. Price, $5.50. Pp. 409. New York: The Macmillan Company, 1935.